SUBSTITUTE SPECIFICATION

ENDOZEPINE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES ENCODING SAME

RELATED APPLICATIONS

This application claims priority to USSN 60/157,786, filed October 5, 1999; USSN 60/164,164, filed November 9, 1999; USSN 60/174,505, filed January 4, 2000; USSN 60/183,859, filed February 22, 2000; USSN 60/190,740, filed March 20, 2000, USSN 60/191,133, filed March 22, 2000, USSN 60/206,006, filed May 19, 2000, USSN 60/215,684, filed June 30, 2000, USSN 60/219,490, filed July 20, 2000, and USSN 60/227,072, filed August 22, 2000. The contents of these applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides encoded therefrom. More specifically, the invention relates to nucleic acids encoding endozepine-like polypeptides , as well as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides.

BACKGROUND OF THE INVENTION

In the developed world there are over 250 million individuals who suffer from disorders associated with nutritional excess. Obesity, Type II diabetes, and the metabolic syndrome X have reached epidemic proportions. The problems associated with these disorders such as hyperlipidemia, hypertension, vascular disease, stroke and end-organ damage exact a huge financial burden to afflicted individuals and society at large. Thus, new pharmacologic approaches to regulate energy metabolism would be of significant medical benefit. The present invention contains a novel family of endozepine polypeptides that offer new therapeutic opportunities to regulate metabolism through their modulation of insulin secretion, insulin sensitivity, glucose and fatty acid utilization and other endocrine functions.

Endozepines are a family of proteins whose members have been reported to have diverse biological effects. These effects can include modulation of gamma-aminobutyric acid (GABA) receptors, insulin homeostasis, and regulation of mitochondrial steriodogenesis. Modulation of GABA receptors, which are present in brain, is thought to modulate pathological anxiety.

Members of the endozepine family include diazepam binding inhibitor (DBI). DBI, or a derivative of DBI, is thought to down-regulate the effects of GABA. DBI is also reported to inhibit both the early and the late phases of glucose-induced insulin release from the isolated perfused rat pancreas.

Several mammalian DBI polypeptides have been described. Mammalian DBIs tend to be highly conserved at their carboxy termini. A human DBI polypeptide of approximately 11 kilodaltons (kD) has been described. This polypeptide has been shown to displace β-carbolines and benzodiazepines bound to brain membrane fractions *in vitro*.

DBI polypeptides have been reported to be present in both brain and non-brain tissue, including gut. It has been proposed that DBI may belong to a new family of gut polypeptides that inhibit glucose-mediated insulin release by hormonal, neurocrine mechanisms, or both.

SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of novel nucleic acid sequences encoding polypeptides related to known endozepines. Nucleic acids encoding endozepine-like polypeptides and derivatives and fragments thereof, will hereinafter be collectively designated as "ENDOX".

In one aspect, the invention provides an isolated ENDOX nucleic acid molecule encoding an ENDOX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acid sequence of human endozepine mRNA. In some embodiments, the ENDOX nucleic acid molecule can hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of an endozepine nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes an ENDOX polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 85% identical to a polypeptide comprising the amino acid sequences of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of an ENDOX nucleic acid (*e.g.*, SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48) or a complement of said oligonucleotide.

Also included in the invention are substantially purified ENDOX polypeptides (SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49). In some embodiments, the ENDOX polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of human endozepine polypeptide.

The invention also features antibodies that immunoselectively-binds to ENDOX polypeptides.

In another aspect, the invention includes pharmaceutical compositions which include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, an ENDOX nucleic acid, an ENDOX polypeptide, or an antibody specific for an ENDOX polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes an ENDOX nucleic acid, under conditions allowing for expression of the ENDOX polypeptide encoded by the DNA. If desired, the ENDOX polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of an ENDOX polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the ENDOX polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of ENDOX.

Also included in the invention is a method of detecting the presence of an ENDOX nucleic acid molecule in a sample by contacting the sample with an ENDOX nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to an ENDOX nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of an ENDOX polypeptide by contacting a cell sample that includes the ENDOX polypeptide with a compound that binds to the ENDOX polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic

acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a Therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, and the various dyslipidemias. The Therapeutic can be, *e.g.*, an ENDOX nucleic acid, an ENDOX polypeptide, or an ENDOX-specific antibody, or biologically-active derivatives or fragments thereof.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, and the various dyslipidemias. The method includes contacting a test compound with an ENDOX polypeptide and determining if the test compound binds to said ENDOX polypeptide. Binding of the test compound to the ENDOX polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, and the various dyslipidemias, by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by an ENDOX nucleic acid. Expression or activity of ENDOX polypeptide is then measured in the test animal, as is expression or activity of the protein in a control animal which recombinantly-expresses ENDOX polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of ENDOX polypeptide in both the test animal and the control animal is compared. A change in the activity of ENDOX polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of an ENDOX polypeptide, an ENDOX nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the ENDOX polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the ENDOX polypeptide present in a control sample. An alteration in the level of the ENDOX polypeptide

in the test sample as compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, and the various dyslipidemias. Also, the expression levels of the new endozepines of the invention can be used in a method to screen for various cancers.

In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject an ENDOX polypeptide, an ENDOX nucleic acid, or an ENDOX-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, and the various dyslipidemias..

In a further aspect, the invention includes a method to alter global energy metabolism or weight loss by altering in serum cholesterol, lipids, glucose and insulin.

In yet another aspect, the invention includes a method to modulate weight loss due to specific adipose deposit reduction, muscle mass increases associated with some medical treatments, or modulation of lipid volume in some adipocytes

In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion. The invention can also be used in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

In yet another aspect, the invention can be used in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described

below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative purposes only, and not intended to be limiting in any manner. Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows 6 photomicrographs deoicting mesenteric adipose deposits in mice in response to treatment.

FIG. 2 shows PCR products of endozepine coding sequences.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode polypeptides related to previously described polypeptides in the endozepine protein family. Nucleic acids encoding endozepine-like polypeptides based on the discovered sequences are referred to individually as ENDO1, ENDO2, ENDO3, ENDO4, ENDO5, ENDO6, ENDO7, ENDO8, ENDO9, and ENDO10. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "ENDOX".

ENDO1

An ENDO1 nucleic acid of the invention comprises nucleic acid sequences shown in SEQ ID NOs: 1, 11, 13, 14, 46, and 48. SEQ ID NOs: 11, 13, and 14 can be combined to provide the nucleotide sequence of SEQ ID NO:1. SEQ ID NO:1 may not be a complete coding sequence as it does not have a starting methioine, and it has no stop codon. SEQ ID NOs: 46 and 48 both encompass nucleotide sequences which encode an amino acid sequence which is also encoded by SEQ ID NO:1. Each of these nucleotide sequences and the amino acids which they encode are described in detail before.

An ENDO1 nucleic acid of the invention includes the nucleic acid sequence shown in Table 1 (SEQ ID NO:11). The sequence is related to an expression sequence tag (EST) from a previously described human cDNA clone, with database accession number AA877351. The AA877351 EST is reported to be similar to a nucleic acid encoding "diazepam binding inhibitor-like 5".

Table 1

ACCGCCTCCACCACCCCATGTGCCAAGTGGAGTTCGAGCTGCGCGGCCCTCAAGCAGCTGAAG
GGTCCCGTGAGCGATCAGGAGAAGCTGCTGGTCTACGGCTTGTACAAACAGGCCACCCAGGGC
GACTGCGACATCCCCGGCCCTCCGGCCTCAGACGTGAGAGCCAGGGCCAAGTGGGAGGCTTGG
AGCGCGAACAAAGGGGCGTCCAAGATGGACGCCATGAGGGGCTACGCGGCCAAAGTGGAGGAG
CTGACGAAGAAGGAA (SEQ ID NO:11)

The nucleic acid sequence disclosed in Table 1 includes an open reading frame ("ORF") beginning at position 1. The ORF encodes a polypeptide sequence of 89 amino acid residues. The sequence of this encoded polypeptide is presented in Table 2 (SEQ ID NO:12). The translated protein is related (Identities 62/81; 76%) and Positives = 70/81; 86%) to diazepam binding inhibitor-like 5(SWISSPROT-ACC:O09035) from mouse. Also, the translated protein is related (Identities = 89/89 (100%), Positives = 89/89 (100%)) to Homo sapiens endozepine-like protein type 2 mutant (GENBANK-ID:AF229804|acc:AF229804).

Table 2

TASTTPCAKWSSSCAALKQLKGPVSDQEKLLVYGLYKQATQGDCDIPGPPASDVRARAKWEAW
SANKGASKMDAMRGYAAKVEELTKKE (SEQ ID NO:12)

The polypeptide encoded by the ORF present in SEQ ID NO:11 does not contain an amino terminal methionine residue. Therefore, the disclosed nucleic acid sequence may be a portion of an open reading frame encoding a larger gene product. The larger gene product may include, for example, at least a signal peptide that is cleaved during protein processing.

To identify additional sequences encoding an ENDO1 nucleic acid, the disclosed ENDO1 sequence (SEQ ID NO:11) was used to design primers for use in PCR reactions to isolate additional sequences encoding the ENDO1 polypeptide shown in Table 2. The amplified sequences were cloned and sequenced. The sequences of two products were named 18517852-2 (SEQ ID NO:13) and 118517852-3 (SEQ ID NO:14). These sequences are shown in Tables 3 and 4, respectively.

Table 3

TCTTCTTCGTCAGCTCCTCCACTTTGGCCGCGTAGCCCCTCATGGCGTCCATCTTGGACGCCCCTTTGTTCGCGCT
CCAAGCCTCCCACTTGGCCCTGGCTCTCACGTCTGAGGCCGGAGGGCCGGGGATGTCGCAGTCGCCCTGGGTGGCC
TGTTTGTACAAGCCGTAGACCAGCAGCTTCTCCTGATCGCTCACGGGACCCTTCAGCTGCTTGAGGGCCGCAAAGC
TCGAACTCCACTTGGCACATGGGGTGGTGGAGGCGGTCCCTGGTGCTAGAAGCTGGAGGTGGAGAGTTGGAGTGGC
TGTTACTACTCGATCTCAGGGGGAGGAGACAGGCACGCGATGTTTGTGTTTTGTCAAGCACAGATTGCAAGCTCGG
GGTCCAGCGTAAACCCCACCATGTTTGGGCTCACACGGCGCATTTTCTGGGGAGGACCAGCCGTCAAAAAGCGTCT
AGGATCCGGAACGCTGCTGTCTGGA (SEQ ID NO:13)

Table 4

GCGTCCATCTTGGACGCCCCTTTGTTCGCGCTCCAAGCCTCCCACTTGGCCCTGGCTCTCACGTCTGAGGCCGGAG
GGCCGGGGATGTCGCAGTCGCCCTGGGTGGCCTGTTTGTACAAGCCGTAGACCAGCAGCTTCTCCTGATCGCTCAC
GGGACCCTTCAGCTGCTTGAGGGCCGCGCAGCTCGAACTCCACTTGGCACATGGGGTGGTGGAGGCGGTCCCTGGT
GCTAGAAGCTGGAGGTGGAGAGTTGGAGTGGCTGTTACTACTCGC (SEQ ID NO:14)

The sequences shown in Tables 1, 3, and 4 can be combined to provide the nucleotide sequence shown in Table 5 (SEQ ID NO:1).

Table 5

GAT CGA GTA GTA ACA GCC ACT CCA ACT CTC CAC CTC CAG CTT CTA GCA CCA GGG ACC GCC TCC
ACC ACC CCA TGT GCC AAG TGG AGT TCG AGC TXT GCG GCC CTC AAG CAG CTG AAG GGT CCC GTG
AGC GAT CAG GAG AAG CTG CTG GTC TAC GGC TTG TAC AAA CAG GCC ACC CAG GGC GAC TGC GAC
ATC CCC GGC CCT CCG GCC TCA GAC GTG AGA GCC AGG GCC AAG TGG GAG GCT TGG AGC GCG AAC
AAA GGG GCG TCC AAG ATG GAC GCC ATG AGG GGC TAC GCG GCC AAA GTG GAG GAG CTG ACG AAG
AAG (SEQ ID NO:1)

The nucleotide residue denoted by "X" can be T or G , *i.e.*, in various embodiments an ENDO1 nucleic acid of the invention includes a T at the position denoted by X in the nucleic acid sequence. In other embodiments, an ENDO1 nucleic acid sequence of the invention includes a G at the position denoted by X in the disclosed nucleotide sequence.

The polypeptide encoded by the ORF in SEQ ID NO:1 does not contain an amino terminal methionine residue. Therefore, the disclosed nucleic acid sequence may be a portion of an open reading frame encoding a larger gene product. To identify additional sequences encoding an ENDO1 nucleic acid, nucleic acid database searches were conducted. Two new ENDO1 sequences have now been indentified and are shown in Table 5A. SEQ ID NO:46 was compiled from a previously described human clone with GenBank AccNo AL121672 (SEQ ID NO:46). SEQ ID NO:48 was compiled from a previously described human clone with GenBank clone AC025743 (SEQ ID NO:48).

Table 5A

>AL121672_GENSCAN_predicted_CDS_5_687_bp

ATGGGAGACGCAGGAGCCACGGCGGCCGCGCTTAGGCCTGCTCACAACCTCCGCCCGGCCCCGCCCACAG
CCTCCGCCGCGCACGCGCAGTCCTCACGAACGAGCGCGCCAAGCGCACAGCGCCGCCTTCCGGCAGAGCC
CTCCCACCAGCCCTCAGCACCAGGGACCGCCTCCACCACCCCATGTGCCAAGTGGAGTTCGAGCTGCGCG
GCCCTCAAGCAGCTGAAGGGTCCCGTGAGCGATCAGGAGAAGCTGCTGGTCTACGGCTTGTACAAACAGG
CCACCCAGGGCGACTGCGACATCCCCGGCCCTCCGGCCTCAGACGTGAGAGCCAGGGCCAAGTGGGAGGC
TTGGAGCGCGAACAAAGGGGCGTCCAAGATGGACGCCATGAGGGGCTACGCGGCCAAAGTGGAGGAGCTG
ACGAAGAAGGAAGTGGGGGGCGTGGAGCGCGAACAAAGGGGCGTGCAAGATGGACGCCATGAGGGGCTAC
GCGGCCAAAGTGGAGGAGCTGACGAAGAAGGAAGGGCGTCCAAGATGGACGCCATGAGGGGCTACGCGGC
CAAAGTGGAGGAGCTGACGAAGAAGGAAGTGGGGGGCGTGGAGCGCGAACAAAGGGGCGTCCAAGATGGA
CGCCATGAGGGGCTACGCGGCCAGAGTGAGGAGATGAGGAAGAAGGAGGCTGGCTGA (SEQ ID NO:46)

>AC025743_GENSCAN_predicted_CDS_7_576_bp

ATGGGAGACGCAGGAGCCACGGCGGCCGCGCTTAGGCCTGCTCACAACCTCCGCCCGGCCCCGCCCACAG
CCTCCGCCGCGCACGCCAGTCCTCACGAACGAGCGCGCCAAGCAAGCCGCGCCTTCCGGCAGAGCCCTCC
CACCAGCCCTCAGCTTCTAGCACCAGGGACCGCCTCCACCACCCCATGTGCCAAGTGGAGTTCGAGCTGC
GCGGCCCTCAAGCAGCTGAAGGGTCCCGTGAGCGATCAGGAGAAGCTGCTGGTCTACGGCTTGTACAAAC
AGGCCACCCAGGGCGACTGCGACATCCCCGGCCCTCCGGCCTCAGACGTGAGAGCCAGGGCCAAGTGGGA
GGCTTGGAGCGCGAAAAAAGGGGCGTCCAAGATGGACGCCATGAGGGGCTACGCGGCCAAAGTGGAGGAG
CTGACGAAGAAGGAAGTGGGGGGCGTGGAGCGCGAACAAAGGGGCGTGCAAGATGGACGCCATGAGGGGC
TACGCGGCCAAAGTGGAGGAGCTGACGAAGAAGGAAGTGGGGGGCGTGGAGCGCGAACAAAGGGGCGTCC
AAGATGGACGCCATGA (SEQ ID NO:48)

The nucleic acid shown in Table 5 (SEQ ID NO:1) encodes a polypeptide having the amino acid sequence shown in Table 6 (SEQ ID NO:2).

Table 6

DRVVTATPTLHLQLLAPGTASTTPCAKWSSSXAALKQLKGPVSDQEKLLVYGLYKQATQGDCD
IPGPPASDVRARAKWEAWSANKGASKMDAMRGYAAKVEELTKKE(SEQ ID NO:2)

The nucleic acids shown in Table 5A (SEQ ID NO:46 and 48) encode polypeptides having the amino acid sequences shown in Table 6A (SEQ ID NO:47 and 49, respectively).

Table 6A

>AL121672_GENSCAN_predicted_peptide_5_228_aa

MGDAGATAAALRPAHNLRPAPPTASAAHAQSSRTSAPSAQRRLPAEPSHQPSAPGTASTTPCAKWSSSCA
ALKQLKGPVSDQEKLLVYGLYKQATQGDCDIPGPPASDVRARAKWEAWSANKGASKMDAMRGYAAKVEEL
TKKEVGGVEREQRGVQDGRHEGLRGQSGGADEEGRASKMDAMRGYAAKVEELTKKEVGGVEREQRGVQDG

RHEGLRGQSEEMRKKEAG(SEQ ID NO:47)

>AC025743_GENSCAN_predicted_peptide_7_191_aa
MGDAGATAAALRPAHNLRPAPPTASAAHASPHERARQASRAFRQSPPTSPQLLAPGTASTTPCAKWSSSC
AALKQLKGPVSDQEKLLVYGLYKQATQGDCDIPGPPASDVRARAKWEAWSAKKGASKMDAMRGYAAKVEE
LTKKEVGGVEREQRGVQDGRHEGLRGQSGGADEEGSGGRGARTKGRPRWTP (SEQ ID NO:49)

The amino acid residue denoted by "X" can be C or F, *i.e.*, in various embodiments a polypeptide of the invention will include a C at the position denoted by X in the amino acid sequence. In other embodiments, an ENDO1 polypeptide of the invention will include an F at the position denoted by X in the recited amino acid sequence.

An ENDO1 nucleic acid of the invention can include a nucleic acid encoding the polypeptide of SEQ ID NO:2,12, 47, or 49, *e.g.*, the ENDO1 nucleic acid can include the nucleic acid sequence of SEQ ID NO:1, 11, 46, or 48. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 1 or Table 5. In some embodiments, the ENDO1 nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO1 polypeptide of the invention can include the amino acid sequence of SEQ ID NO: 2,12, 47, or 49. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:2,12, 47, or 49, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptide

(SEQ ID NO:15)

Metabolism-Regulating Peptide #6 (MRP-6) Sequence:

QATQGDCDIPGPPASDVRAR (SEQ ID NO:15)

A multiple sequence alignment of various embodiments of the ENDO1 polypeptide (Table 6B) displays their relationship to one another.

Table 6B

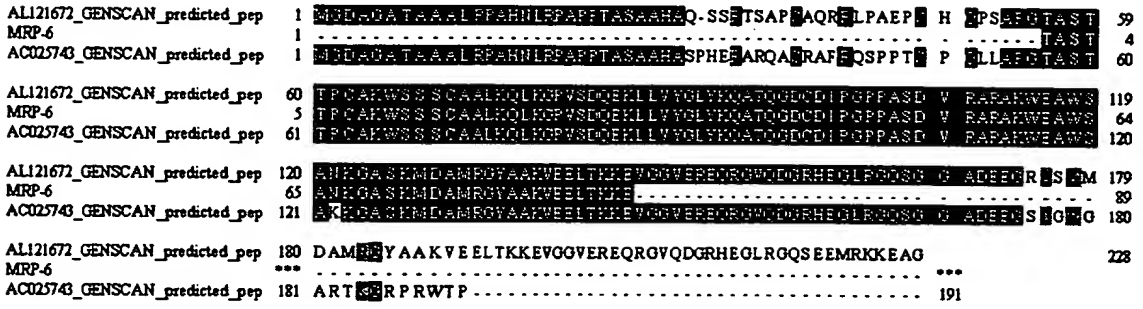

Table 6B-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 6B.

Table 6B-1

SEQUENCE IDENTIFIER	SEQ ID NO
AL121672_GENSCAN_predicted_pep	SEQ ID NO: 150
MRP-6	SEQ ID NO: 149
AC025743_GENSCAN_predicted_pep	

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO1 polypeptide, and derivatives and fragments, thereof.

An ENDO1 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO1 is highly expressed in liver and endothelial cells. Also, high expression of ENDO1 is a marker for multiple types of cancer.

An ENDO1 sequence is also useful to modulate global energy metabolism or weight by altering serum glucose or adipose.level

An ENDO1 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO1 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO2

An ENDO2 nucleic acid of the invention includes the nucleic acid sequence shown in Table 7 (SEQ ID NO:3). An ORF, as well as putative untranslated regions upstream from the initiation codon and downstream from the stop codon of the ORF, is present in the nucleotide sequence disclosed in Table 7. Untranslated nucleotides are shown by underlining. The start and stop codons of the ORF are shown in bold letters.

Table 7

GTATAAGACATACAGAAGGAATGCCTGGAGAGCAGCAACAGCCCAGCTGCGGCCACC**ATG**TCC
CTGCAGGCTGATTTTGACATGGTCACAGAAGATGTGAGGAAGCTGAAAACAAGACCAGATGAT
GAAGAACTGAAAGAACTTTATGGGCTTTACAAACAAGCTGTAATTGGAAACATTAATATTGAG
TGTTCAGAAATGCTAGAATTAAAAGGCAAGGCCAAATGGGAAGCACAGAACCCCCAAAAAGGA
TTGTCAGAGGAAGATATGATGCGTGCCTTTATTTCTAAAGCCGAAGAGCTGATAGAAAAATAT
GGAATT**TAG**AATAAAGCATATGATAAATTTTCCTTT (SEQ ID NO:3)

The nucleic acid sequence of Table 7 has 218 of 294 bases (74%) identical and positive to a 470 nucleotide *Rana ridibunda* diazepam-binding inhibitor (DBI) mRNA (GENBANK-ID: RRU09205|acc:U09205). A BLASTN identify search comparing regions of the sequence disclosed in Table 7 to the *Rana ridbunda* DPI mRNA is shown in Table 8. Regions of the disclosed sequence is are presented as "Query" sequences, and the *Rana ridbunda* DPI mRNA (SEQ ID NO:11) sequences are presented as the "Subject sequences".

Table 8

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Query:    46 GCTGCGGCCACCATGTCCCTGCAGGCTGATTTTGACATGGTCA-CAGAAGATGTGAGGAA
104
           |||| | |||||||| | ||||| |||||||||| | || ||| ||||| | |||
Sbjct:     1 GCTGAATCAACCATGTCACCCCAGGCAGATTTTGACAAAG-CAGCAGGGGATGTAAAGAA 59

Query:   105 GCTGAAAACAAGACCAGATGATGAAGAACTGAAAGAACTTTATGGGCTTTACAAACAAGC
164
             ||||||||| |||| ||| || |||||||| ||||| || || || ||||| ||  |
Sbjct:    60 ATTGAAAACAAAACCAACTGACGATGAACTGAAGGAACTGTACGGACTCTACAAGCAGTC
119

Query:   165 TGTAATTGGAAACATTAATATTGAGTGTTCAGAAATGCTAGAATTAAAAGGCAAGGCCAA
224
               ||||  |||| ||||| |||||| | |  |||||||  | || ||||||||||
Sbjct:   120 CACTGTTGGGGACATAAATATAGAGTGTCCTGGCATGCTAGATCTGAAGGGCAAGGCCAA
179

Query:   225 ATGGGAAGCACAGAACCCCCAAAAAGGATTGTCAGAGGAAGATATGATGCGTGCCTTTAT
284
             ||||| |||  |||||   | ||||| |||||  ||||||||  |||| | || | | |
Sbjct:   180 GTGGGACGCATGGAACCTAAAGAAAGGCTTGTCTAAGGAAGATGCGATGAGCGCTTATGT
239

Query:   285 TTCTAAAGCCGAAGAGCTGATAGAAAAATATGGAATTTAGAATAAAG-CATATGAT 339
(SEQ ID NO:50)
             |||||||||| | |||||||||||||||||||  | || |  || | |  |||||
Sbjct:   240 TTCTAAAGCCCATGAGCTGATAGAAAAATATGGCCTGTA-AC-AAGGTCGCATGAT 293
(SEQ ID NO:51)
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The ORF encodes a polypeptide of 85 amino acids (SEQ ID NO:4). The amino acid sequence of this polypeptide is shown in Table 9 (SEQ ID NO:4).

Table 9

MSLQADFDMVTEDVRKLKTRPDDEELKELYGLYKQAVIGNINIECSEMLELKGKAKWEAQNPQ
KGLSEEDMMRAFISKAEELIEKYGI (SEQ ID NO:4)

The polypeptide sequence disclosed in Table 9 is related to a previously described duck diazepam binding inhibitor polypeptide. This relationship is shown in Table 10. The amino acid sequence shown in Table 9 (SEQ ID NO:4) has 60 of 85 amino acid residues (70 %) identical to, and 72 of 85 residues (84%) positive with, the 103 amino acid residue acyl-coA-binding protein (ACBP) (diazepam binding inhibitor)(DBI) (endozepine) (EP) from *Anas platyrhynchos* (domestic duck) (ptnr: SWISSPROT-ACC:P45882). Regions of the

polypeptide sequnce shown in Table 9 are presented as the "Query" sequence. Regions of the duck polypeptide sequence are shown as the "Sbct" sequences.

Table 10

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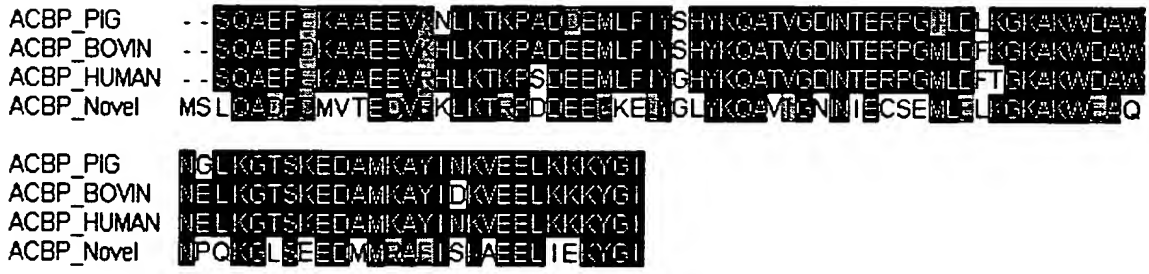
Query:    67 QADFDMVTEDVRKLKTRPDDEELKELYGLYKQAVIGNINIECSEMLELKGKAKWEAQNPQ
5   246
                |||||   |+|+|||||| ||||||||| |||| +|+|||||  ||+|||||||| | +
Sbjct:    19 QADFDEAAEEVKKLKTRPTDEELKELYGFYKQATVGDINIECPGMLDLKGKAKWEAWNLK 78

Query:   247 KGLSEEDMMRAFISKAEELIEKYGI 321 (SEQ ID NO:52)
10              ||+|+|| | |+|||+ ++|||||
Sbjct:    79 KGISKEDAMNAYISKAKTMVEKYGI 103 (SEQ ID NO:53)
```

A multiple sequence alignment between the amino acids of SEQ ID NO:4 and various acyl coA binding polypeptides is illustrated in Table 11. Shown is an alignment between the
15 amino acid sequence of Table 7 ("ACBP_Novel"), porcine acyl-coA binding protein (SWISSPROT locus ACBP_PIG, accession No. P12026) ("ACBP_PIG"), bovine acyl-coA binding protein (SWISSPROT locus ACBP_BOVIN, accession no. P07107)("ACBP_BOVIN"), and human acyl-coA binding protein (SWISSPROT locus ACBP_HUMAN, accession no. P07108)("ACBP_HUMAN"). Regions of perfect homology
20 are shown in black. Regions with conservative amino acid substitutions are shown in gray. Non-conservative amino acid substitutions are presented without shading.

Table 11



25

Table 11-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 11.

Table 11-1

SEQUENCE IDENTIFIER	SEQ ID NO
ACBP_PIG	SEQ ID NO: 54
ACBP_BOVIN	SEQ ID NO: 55
ACBP_HUMAN	SEQ ID NO: 56
ACBP_Novel	SEQ ID NO: 57

An ENDO2 nucleic acid of the invention encoding a endozepine-like protein includes the nucleic acid encoding a polypeptide that includes the amino acid sequence of SEQ ID NO:4, *e.g.*, a nucleic acid whose sequence is provided in SEQ ID NO:3. The invention also includes a fragment of SEQ ID NO:3, or a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in SEQ ID NO:3. In some embodiments, the mutant or variant nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO2 polypeptide according to the invention includes a polypeptide comprising the an amino acid sequence shown in Table 9 (SEQ ID NO:4). The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 8, while still encoding a protein that maintains its endozepine-like

activities and physiological functions, or a functional fragment thereof such as the following active peptide (SEQ ID NO: 16)

Metabolism-Regulating Peptide #5 (MRP-5) Sequence:

QAVIGNINIECSEMLELKGK (SEQ ID NO: 16).

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO2 polypeptide, and derivatives and fragments, thereof.

An ENDO2 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO2 is highly expressed in brain and pancreas cells. Also, high expression of ENDO2 is a marker for colon and lung cancer.

An ENDO2 sequence is also useful to modulate global energy metabolism or weight by altering serum glucose or adipose level.

An ENDO2 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO2 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO3

An ENDO3 nucleic acid of the invention includes the nucleic acid sequence shown in Table 12 (SEQ ID NO:5). An ORF, as well as putative untranslated regions upstream from the initiation codon and downstream from the stop codon of the ORF, are shown in Table 12 by underlining. The start and stop codons of the ORF are shown in bold letters. The ORF

begins with an atg initiation codon at nucleotides 86-88 and ends with a tga codon at nucleotide 403-405. Putative untranslated regions upstream from the initiation codon and downstream from the stop codon are shown in Table 12 by underlining, whereas the start and stop codons are shown in bold letters.

Table 12

GCTCACACCTGTAATCCCAGCATTTGGGAGGCCAAGGCAGGCAGATTATGTGAGGTCAAGAGT
TCCAGACCAGCTGTCCAAC**ATG**GCAAAACCCATCTCCACTAAAAATACAAAAATTAGCCGGCA
TGGGTGGCATGCAGCTGTAATCACAGCTGCTCGGGAGGCTGAGGCGGAGAATCACTTGAGCTG
GGAAGAAAAAAAAAAAAAAAAAAGATGTGCAGGTATTAAGCACTTTAAGACCAAGCCAGCAGA
TGATGAGATGCGGTTCCTTTACGGCCACTACAAACGAGCGACTGTAGGCAACATAAAGACAGA
ACGGCCAGGGATGGTGGACTTCAAGGGCAAAGCCAAGTGGGATCCCTGGAATTTAGTGAAAGG
GGCTGCCAGGGAAGATCCCATGAAAGCTAAAGCTTACGTCAAAAAAGTAGAAGAGTTAAAGAA
AAAATTCAGAATACGAGAGACTGGAATTGTTGCCAGCCATGCCTTTGTCCTAAAC**TGA**GACAA
TGCCTTGTTTTTTCTACACTGTGGATGGTGGGAACTGATGGAAAGAATCAGCTAACCCATC
(SEQ ID NO:5)

The disclosed ENDO3 nucleic acid sequence (SEQ ID NO:5) has 168 of 199 bases (84%) identical to a sequence on human chromosome 16 incorporated into bacterial artificial chromosome 462G18 (LANL) (GENBANK-ID: AC005736|acc:AC005736).

The ORF identified in Table 12 encodes a polypeptide sequence of 83 residues (SEQ ID NO:6), which is presented in Table 13.

Table 13

MAKPISTKNTKISRHGWHAAVITAAREAEAENHLSWEEKKKKKRCAGIKHFKTKPADDEMRFL
YGHYKRATVGNIKTERPGMVDFKGKAKWDPWNLVKGAAREDPMKAKAYVKKVEELKKKFRIRE
TGIVASHAFVLN (SEQ ID NO:6)

The amino acid sequence of the polypeptide sequence disclosed in Table 13 (SEQ ID NO:6) has 55 of 82 amino acid residues (67%) identical to, and 66 of 82 residues (80%) positive with, the 86 amino acid residue bovine acyl-coA-binding protein (ACBP) (diazepam binding inhibitor)(DBI) (endozepine) (EP) (ptnr: SWISSPROT-ACC: P07107). A comparison of these sequences is presented in Table 14. Regions of the polypeptide of Table 13 are

presented as the "Query" sequence, and regions of the bovine ACBP sequence are presented as the "Sbjct" sequence.

Table 14

```
Query:   209 KRCAGIKHFKTKPADDEMRFLYGHYKRATVGNIKTERPGMVDFKGKAKWDPWNLVKGAAR
388
                |    +|| ||||||+|| |+| |||+||||+| ||||||+|||||||| || +|| ++
Sbjct:     7 KAAEEVKHLKTKPADEEMLFIYSHYKQATVGDINTERPGMLDFKGKAKWDAWNELKGTSK 66

Query:   389 EDPMKAKAYVKKVEELKKKFRI 454 (SEQ ID NO:58)
                || |||  |+ |||||||+ |
Sbjct:    67 EDAMKA--YIDKVEELKKKYGI 86 (SEQ ID NO:59)
```

The amino acid sequence of the polypeptide sequence disclosed in Table 13 (SEQ ID NO:6) has 57 of 91 amino acid residues (62%) identical to, and 72 of 91 residues (79%) positive with 91 amino acid residues of Human diazepam binding inhibitor (DBI) (gb:GENBANK-ID:HUMDBI|acc:M14200)

A comparison of these sequences is presented in Table 14A. Regions of the polypeptide of Table 13 are presented as the "Query" sequence, and regions of the Human diazepam binding inhibitor (DBI) sequence are presented as the "Sbjct" sequence.

Table 14A

```
>gb:GENBANK-ID:HUMDBI|acc:M14200 Human diazepam binding inhibitor (DBI)
mRNA, complete cds - Homo sapiens, 556 bp.
Length = 556

Plus Strand HSPs:

Score = 310 (109.1 bits), Expect = 3.4e-26, P = 3.4e-26
Identities = 57/91 (62%), Positives = 72/91 (79%), Frame = +2

Query:    38 EKKKKKRCAGIKHFKTKPADDEMRFLYGHYKRATVGNIKTERPGMVDFKGKAKWDPWNLV 97
             | + +|    ++| ||||+|+|| |+|||||+||||+| ||||||+|| |||||| || +
Sbjct:    77 EAEFEKAAEEVRHLKTKPSDEEMLFIYGHYKQATVGDINTERPGMLDFTGKAKWDAWNEL
256

Query:    98 KGAAREDPMKAKAYVKKVEELKKKFRIRETG 128 (SEQ ID NO:60)
             || ++|| |||  |+ |||||||+ | |||
Sbjct:   257 KGTSKEDAMKA--YINKVEELKKKYGI*ETG 343 (SEQ ID NO:61)
```

A multiple sequence alignment illustrating the relatedness of the polypeptide disclosed in Table 13 to bovine and human Acyl co-A binding proteins is presented in Table 15 as a

ClustalW analysis. Compared are the polypeptide of Table 12 ("DBI_novel"), bovine acyl coA-binding protein (SWISSPROT locus ACBP_BOVIN, accession P07107) ("ACBP_bovin"), and ACBP_human SWISSPROT locus ACBP_HUMAN, accession _ P07108 ("ACBP_Human"). Regions of perfect homology are shown in black. Regions with conservative amino acid substitutions are shown in gray. Non-conservative amino acid substitutions are presented without shading.

Table 15

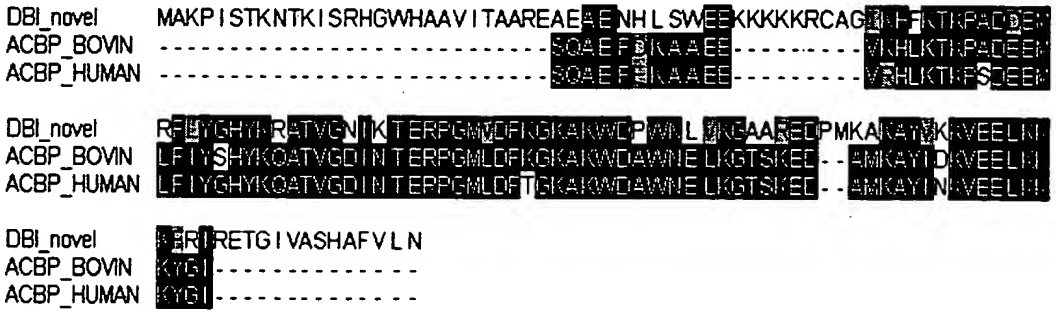


Table 15-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 15.

Table 15-1

SEQUENCE IDENTIFIER	SEQ ID NO
DBI_novel	SEQ ID NO: 62
ACBP_BOVIN	SEQ ID NO: 63
ACBP_HUMAN	SEQ ID NO: 64

An ENDO3 nucleic acid of the invention includes a nucleic acid encoding a polyeptide comprising SEQ ID NO:6, *e.g.*, a nucleic acid whose sequence is shown in SEQ ID NO:5. The invention also includes a fragment of the nucleic acid of SEQ ID NO:5, as well as a mutant or variant nucleic acid, any of whose bases may be changed from the corresponding base shown in Table 12, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just

described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO3 protein of the invention includes the amino acid sequence shown in Table 13 (SEQ ID NO:6). The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 13, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptides

Metabolism-Regulating Peptide #3 (MRP-3, 3s) Sequences (SEQ ID NO:17) and : (SEQ ID NO:18)

RATVGNIKTERPGMVDFKGK (SEQ ID NO:17)

RATVGNIKTERPGMVDFK--(SEQ ID NO:18)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO3 polypeptide, and derivatives and fragments, thereof.

An ENDO3 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO3 is highly expressed in adipose and skeletal muscle. Also, high expression of ENDO3 is a marker for breast cancer.

An ENDO3 sequence is also useful to modulate global energy metabolism or weight by altering serum insulin or adipose level.

An ENDO3 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO3 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO4

An ENDO4 nucleic acid of the invention includes the nucleic acid sequence shown in Table 16 (SEQ ID NO:7). The sequence shown in Table 16 includes an ORF, as well as putative untranslated regions upstream and downstream from the ORF. The ORF begins with an atg initiation codon at nucleotides 11-13 and ends with a tga codon at nucleotides 299-301. The putative upstream and downstream untranslated regions are shown by underlining in Table 16.

Table 16

TTGGTGGTAAATGCTCCTTTTGTTTGTTTGTTTGTTCTTCCTTAAGGCTGATTTTGACAGGGC
TGCAGAAGATGTGAGGAAGCTGAAAGCAAGACCAGATGATGGAGAACTGAAAGAACTCTATGG
GCTTTACAAACAAGCAATAGTTGGAGACATTAATATTGCGTGTCCAGGAATGCTAGATTTAAA
AGGCAAAGCCAAATGGGAAGCATGGAACCTCAAAAAAGGGTTGTCGACGGAAGATGCGACGAG
TGCCTATATTTCTAAAGCAAAGGAGCTGATAGAAAAATACGGAATTTAGAATACAGCA (SEQ
ID NO:7)

The disclosed nucleic acid sequence (SEQ ID NO:7) has 200 of 256 bases (78%) identical to a *Rana ridibunda* endozepine mRNA (GENBANK-ID: *RRU09205*| acc:U09205). The relationship between the sequence of Table 16 and the *Rana ridibunda* sequence is presented in Table 17. Regions of the sequence shown in Table 16 are listed as the "Query" sequence. Regions of the *Rana ridibunda* endozepine mRNA sequence are shown as the "Subject" sequence.

Table 17

```
Query:    45 AGGCTGATTTTGACAGGGCTGCAGAAGATGTGAGGAAGCTGAAAGCAAGACCAGATGATG
104

Sbjct:    23 AGGCAGATTTTGACAAAGCAGCAGGGGATGTAAAGAAATTGAAAACAAAACCAACTGACG 82

Query:   105 GAGAACTGAAAGAACTCTATGGGCTTTACAAACAAGCAATAGTTGGAGACATTAATATTG
164

Sbjct:    83 ATGAACTGAAGGAACTGTACGGACTCTACAAGCAGTCCACTGTTGGGGACATAAATATAG
142

Query:   165 CGTGTCCAGGAATGCTAGATTTAAAAGGCAAAGCCAAATGGGAAGCATGGAACCTCAAAA
224
```

```
Sbjct:   143 AGTGTCCTGGCATGCTAGATCTGAAGGGCAAGGCCAAGTGGGACGCATGGAACCTAAAGA
202

Query:   225 AAGGGTTGTCGACGGAAGATGCGACGAGTGCCTATATTTCTAAAGCAAAGGAGCTGATAG
284
             ||||  |||||  | ||||||||||| ||| || ||| ||||||||||  | ||||||||||
Sbjct:   203 AAGGCTTGTCTAAGGAAGATGCGATGAGCGCTTATGTTTCTAAAGCCCATGAGCTGATAG
262

Query:   285 AAAAATACGGAATTTA 300 (SEQ ID NO:65)
             |||||||  ||   | ||
Sbjct:   263 AAAAATATGGCCTGTA 278 (SEQ ID NO:66)
```

The disclosed nucleic acid sequence (SEQ ID NO:7) is also related to a human diazepam binding inhibitor mRNA (GENBANK-ID:HUMDBI|acc:M14200). The disclosed sequence is identical at 179 of 259 residues (69%) to the human diazepam inhibitor mRNA. The relationship between the disclosed and the human sequence is presented in Table 18. Regions of the sequence shown in Table 16 are listed as the "Query" sequence. Regions of the human mRNA sequence are shown as the "Subject" sequence.

Table 18

```
Query:    45 AGGCTGATTTTGACAGGGCTGCAGAAGATGTGAGGAAGCTGAA-AGCAAGACCAGATGAT
103
             |||||||  |||||  |  ||||||||  ||  ||  |||  |  ||  ||  |  ||||  |||    |||
Sbjct:    78 AGGCTGAGTTTGAGAAAGCTGCAGAGGAGGTTAGGCACCTTAAGACCAAG-CCATCGGAT
136

Query:   104 GGAGAACTGAAAGAAC-TCTATGGGCTTTACAAACAAGCAATAGTTGGAGACATTAATAT
162
             |  ||  ||   |  |  |||||||  |  ||||||||||||  ||  ||  |||||  ||||
Sbjct:   137 GAGGAGATGCT-GTTCATCTATGGCCACTACAAACAAGCAACTGTGGGCGACATAAATAC
195

Query:   163 TGCGTGTCCAGGAATGCTAGATTTAAAAGGCAAAGCCAAATGGGAAGCATGGAACCTCAA
222
              |   |  ||  ||  |||  |  ||  ||  |   |||||  |||||  |||||  ||  |||||
Sbjct:   196 AGAACGGCCCGGGATGTTGGACTTCACGGGCAAGGCCAAGTGGGATGCCTGGAATGAGCT
255

Query:   223 AAAAGGGTTGTCGACGGAAGATGCGACGAGTGCCTATATTTCTAAAGCAAAGGAGCTGAT
282
              ||||||    ||  |  |||||||||  |  ||   ||  ||  ||     ||||  |  |  |||||  |
Sbjc|:   256 GAAAGGGACTTCCAAGGAAGATGCCATGAAAGCTTACATCAACAAAGTAGAAGAGCTAA-
314

Query:   283 AGAAAAA-TACGGAATTT-AGA 302 (SEQ ID NO:67)
             |||||||  |||||  ||  |  |||
```

```
Sbjct:   315 AGAAAAAATACGGGATATGAGA 336 (SEQ ID NO:68)
```

The ORF identified in Table 16 encodes an amino acid sequence of 89 amino acids (SEQ ID NO:8). The amino acid sequence of the encoded protein (SEQ ID NO:8) is shown in Table 19.

Table 19

MLLLFVCLFFLKADFDRAAEDVRKLKARPDDGELKELYGLYKQAIVGDINIACPGMLDLKGKA
KWEAWNLKKGLSTEDATSAYISKAKELIEKYGI (SEQ ID NO:8)

A signal peptide is present in the polypeptide sequence shown in Table 19. The most likely cleavage site between residues 18 and 19, at the sequence DRA-AE. The program PSORT predicts a moderate likelihood of extracellular secretion for the ENDO4 protein.

The polypeptide shown in Table 19 is related to previously described acyl-coA binding proteins and diazepam binding inhibitor proteins. Table 20 shows that the amino acid sequence of Table 19 has 71 of 89 amino acid residues (79%) identical to, and 78 of 89 residues (87%) positive with, the 103 amino acid residue protein from *Anas platyrhynchos* (ptnr: SWISSPROT-ACC:P45882). Regions of the polypeptide sequence shown in Table 19 are presented as the "Query" sequence. Regions of the *Anas platyrhynchos* sequence are shown as the "Sbjct" sequence.

Table 20

```
Query:    35 FFL-KADFDRAAEDVRKLKARPDDGELKELYGLYKQAIVGDINIACPGMLDLKGKAKWEA
211
                |||  +||||  |||+|+|||  ||  |  |||||||  ||||  ||||||  ||||||||||||||||
Sbjct:    15 FFLHQADFDEAAEEVKKLKTRPTDEELKELYGFYKQATVGDINIECPGMLDLKGKAKWEA 74

Query:   212 WNLKKGLSTEDATSAYISKAKELIEKYGI 298 (SEQ ID NO:69)
               |||||||+|  |||  +|||||||  ++|||||
Sbjct:    75 WNLKKGISKEDAMNAYISKAKTMVEKYGI 103 (SEQ ID NO:70)
```

Table 20A shows that the amino acid sequence of Table 19 has homology to Rana ridibunda diazepam-binding inhibitor (DBI). Regions of the polypeptide sequence shown in Table 19 are presented as the "Query" sequence. Regions of the Rana ridibunda diazepam-binding inhibitor (DBI)sequence are shown as the "Sbjct" sequence.

Table 20A

```
>gb:GENBANK-ID:RRU09205|acc:U09205 Rana ridibunda diazepam-binding inhibitor
(DBI) mRNA, complete cds - Rana ridibunda, 470 bp.
Length = 470

Plus Strand HSPs:

Score = 365 (128.5 bits), Expect = 6.0e-32, P = 6.0e-32
Identities = 68/85 (80%), Positives = 76/85 (89%), Frame = +1

Query:    12 KADFDRAAEDVRKLKARPDDGELKELYGLYKQAIVGDINIACPGMLDLKGKAKWEAWNLK 71
             +ADFD+AA DV+KLK +P D ELKELYGLYKQ+ VGDINI CPGMLDLKGKAKW+AWNLK
Sbjct:    22 QADFDKAAGDVKKLKTKPTDDELKELYGLYKQSTVGDINIECPGMLDLKGKAKWDAWNLK
201

Query:    72 KGLSTEDATSAYISKAKELIEKYGI 96  (SEQ ID NO:71)
             KGLS EDA SAY+SKA ELIEKYG+     (SEQ ID NO:72)
Sbjct:   202 KGLSKEDAMSAYVSKAHELIEKYGL 276 (SEQ ID NO:73)
```

An alignment between the polypeptide sequence shown in Table 18 and previously described diazepam binding inhibitor or Acyl-coA binding polypeptides is shown in Table 21. The amino acid sequence shown in Table 19 is presented as "ba271m1_A". An 88 amino acid frog acyl co-A binding protein amino acid sequence (PIR-ID: A57711) is indicated by "A57711_ACBP_Frog.: An 88 amino acid human acyl co-A binding polypeptide (PIR-ID: NZHU) sequence is shown by "NZHU_ACBP_Human". A 103 amino acid duck endozepine amino acid sequence (SWISSPROT-ACC:_45882) is indicated by "P45882_endozepine_Duck". Regions with conservative amino acid substitutions are shown in gray. Non-conservative amino acid substitutions are presented without shading.

Table 21

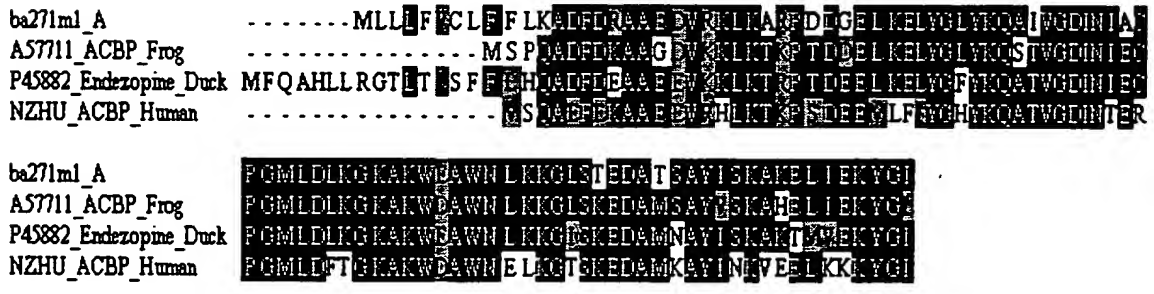


Table 21-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 21.

Table 21-1

SEQUENCE IDENTIFIER	SEQ ID NO
ba271m1_A	SEQ ID NO: 74
A57711_ACBP_Frog	SEQ ID NO: 75
P45882_Endezopine_Duck	SEQ ID NO: 76
NZHU_ACBP_Human	SEQ ID NO: 77

An ENDO4 nucleic acid of the invention includes a nucleic acid encoding a polypeptide that includes the amino acid sequence of SEQ ID NO:8. For example, an ENDO4 nucleic acid can include the sequence disclosed in Table 16 (SEQ ID NO:7). The invention alco includes fragments of a nucleic acid encoding a polypeptide that includes the amino acid sequence of SEQ ID NO:8. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 16, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO4 protein of the invention includes the protein whose amino acid sequence is shown in Table 19 (SEQ ID NO:8). The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 19, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptides

Metabolism-Regulating Peptide #4 (MRP-4,4s) Sequences:

QAIVGDINIACPGMLDLKGK (SEQ ID NO:19)

QAIVGDINIACPGMLDLK--(SEQ ID NO:20)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO4 polypeptide, and derivatives and fragments, thereof.

An ENDO4 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO4 is highly expressed in hematopoietic tissue.

An ENDO4 sequence is also useful to modulate global energy metabolism or weight by altering serum insulin and glucose.

An ENDO4 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO4 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO5

An ENDO5 nucleic acid according to the invention includes the nucleic acid sequence shown in Table 25 (SEQ ID NO:9). An ORF is present in the disclosed sequence, as well as putative untranslated regions upstream and downstream of the ORF. The ORF begins with an atg initiation codon at nucleotides 7-9 and ends with a tag codon at nucleotides 265-267. The putative upstream and downstream untranslated regions are shown by underlining in Table 22.

Table 22

ACCACC**ATG**GCACTGCAGGCTGAATTCGACAAGGCTGCAGAAGACGTGAGGAAGCTGCCAACA
AGACCAGCAGATAATAAAGAACTGAAAAAACTCGATGGACTTTACAAACAAGCTATAATTGGA
GACATTAATATTGAGTATCTGGGAATGCTGGACTTTAAGGGCAAGGCCAAATGCGCAGCATGG
ACCCTCCAAAAAAGGTTGTCAAAGGAAGATGCAACGAGTGTCTCTATTTCTAAGGCAAAAGAG
CCGATAGAAAAA**TAG**GACATTTAGAATA (SEQ ID NO :9)

The ENDO5 nucleic acid sequence (SEQ ID NO:9) has 199 of 274 nucleotides (72%) identical to a *Rana ridibunda* endozepine mRNA GENBANK-ID: RRU09205|acc:U09205. A comparison of these nucleotide sequences is shown in Table 23. The sequence disclosed in Table 22 is presented as the "Query" sequence, and the *Rana ridibunda* endozepine mRNA sequence is presented as as the "Sbjct" sequence.

Table 23

```
Query:     2 CCACCATGGCACTGCAGGCTGAATTCGACAAGGCTGCAGAAGACGTGAGGAAGCTGCCAA 61
             | |||||| |||  ||||| || || ||||| || ||||  || || | |||  ||  ||
Sbjct:     8 CAACCATGTCACCCCAGGCAGATTTTGACAAAGCAGCAGGGGATGTAAAGAAATTGAAAA 67

Query:    62 CAAGACCAGCAGATAATAAAGAACTGAAAAAACTCGATGGACTTTACAAACAAGCTATAA 121
             ||| |||| | ||  ||   ||||||||  ||||  | |||||  |||||  ||  | |
Sbjct:    68 CAAAACCAACTGACGAT---GAACTGAAGGAACTGTACGGACTCTACAAGCAGTCCACTG 124

Query:   122 TTGGAGACATTAATATTGAGTATCTGGGAATGCTGGACTTTAAGGGCAAGGCCAAATGCG 181
             |||| ||||| ||||| |||| ||  || ||||| ||  | ||||||||||||||  || |
Sbjct:   125 TTGGGGACATAAATATAGAGTGTCCTGGCATGCTAGATCTGAAGGGCAAGGCCAAGTGGG 184

Query:   182 CAGCATGGACCCTCCAAAAAAGG-TTGTCAAAGGAAGATGCAACGAGTGTCTCTATTTCT 240
               ||||||| |||  || ||||| ||||| |||||||||||| | ||| |  | | |||||
Sbjct:   185 ACGCATGGAACCTA-AAGAAAGGCTTGTCTAAGGAAGATGCGATGAGCGCTTATGTTTCT 243

Query:   241 AAGGCAAAAGAGCCGATAGAAAAATAGGACATTTA 275 (SEQ ID NO:78)
             || ||  | |||| |||||||||||| | | | ||
Sbjct:   244 AAAGCCCATGAGCTGATAGAAAAATATGGCCTGTA 278 (SEQ ID NO:79)
```

The ENDO5 nucleic acid sequence (SEQ ID NO:9) also has 173 of 262 nucleotides (66%) identical to a *Homo sapiens* endozepine mRNA GENBANK-ID: HUMEDZ|acc:M15887. A comparison of these nucleotide sequences is shown in Table 24. The sequence disclosed in Table 22 is presented as the "Query" sequence, and the human endozepine mRNA sequence is presented as the "Sbjct" sequence.

Table 24

```
Query:    16 CAGGCTGAATTCGACAAGGCTGCAGAAGACGTGAGGAAGCTGCCAAC-AAGACCAGCAGA 74
             |||||||| || || || |||||||| || || ||| | ||    || ||| ||| | ||
Sbjct:    64 CAGGCTGAGTTTGAGAAAGCTGCAGAGGAGGTTAGGCACCTTAAGACCAAG-CCATCGGA 122

Query:    75 TAATAAAGAA-CTGAAAAAACTCGATGGACTTTACAAACAAGCTATAATTGGAGACATTA 133
             | |  | ||  |||   |  ||  |||| |  |||||||||| |   | || ||||| |
Sbjct:   123 TGAGGA-GATGCTGTTCAT-CT--ATGGCCACTACAAACAAGCAACTGTGGGCGACATAA 178
```

```
Query:   134 ATATTGAGTATCTGGGAATGCTGGACTTTAAGGGCAAGGCCAAATGCGCAGCATGGACCC 193
             |||  ||    |  || ||| ||||||| | ||||||||||| || |  || ||||
Sbjct:   179 ATACAGAACGGCCCGGGATGTTGGACTTCACGGGCAAGGCCAAGTGGGATGCCTGGAATG 238

Query:   194 TCCAAAAAAGGTTGTCAAAGGAAGATGCAACGAGTGTCTCTATTTCTAAGGCAAAAGAGC 253
               |  ||| ||   || ||||||||||| | ||  |  |  ||    || | | ||||||
Sbjct:   239 AGCTGAAAGGGACTTCCAAGGAAGATGCCATGAAAGCTTACATCAACAAAGTAGAAGAGC 298

Query:   254 CGATAGAAAAA-TAGGACATTT-AGA 277 (SEQ ID NO:80)
               | ||||||| || |  || | |||
Sbjct:   299 TAA-AGAAAAAATACGGGATATGAGA 323 (SEQ ID NO:81)
```

The ORF identified in Table 22 encodes a polypeptide of 86 amino acid residues (SEQ ID NO:10). The amino acid sequence of the encoded polypeptide is shown in Table 25.

Table 25

MALQAEFDKAAEDVRKLPTRPADNKELKKLDGLYKQAIIGDINIEYLGMLDFKGKAKCAAWTL QKRLSKEDATSVSISKAKEPIEK (SEQ ID NO:10)

The encoded polypeptide (SEQ ID NO:10) shown in Table 25 has 57 of 86 amino acid residues (66%) identical to, and 67 of 86 residues (77%) positive with, the 88 amino acid residue diazepam binding inhibitor protein from *Rana ridibunda* (ptnr:PIR-ID:A57711). An alignment of these sequences is shown in Table 26. A comparison of these amino acid sequences is shown in Table 26. The sequence disclosed in Table 25 is presented as the "Query" sequence, and the *Rana ridibunda* endozepine polypeptide sequence is presented as the "Sbjct" sequence.

Table 26

```
Query:     7 MALQAEFDKAAEDVRKLPTRPADNKELKKLDGLYKQAIIGDINIEYLGMLDFKGKAKCAA 186
             |+ ||+|||||  ||+|| |+| |+ |||+| |||||+ +||||||  |||| |||||  |
Sbjct:     1 MSPQADFDKAAGDVKKLKTKPTDD-ELKELYGLYKQSTVGDINIECPGMLDLKGKAKWDA 59

Query:   187 WTLQKRLSKEDATSVSISKAKEPIEK 264 (SEQ ID NO:82)
             | |+| |||||| |  +||| | |||
Sbjct:    60 WNLKKGLSKEDAMSAYVSKAHELIEK 85 (SEQ ID NO:83)
```

An alignment showing the relatedness of the polypeptide sequence shown in Table 25 to previously described endozepine sequences is shown in Table 27. The 86 amino acid polypeptide of Table 25 is shown as "citb_e1_2540m10_A". The other endozepine polypeptide sequences present in the table include 88 amino acid sequence of frog diazepam

binding inhibitor DBI (PIR-ID:A57711) ("A57711"), a 103 amino acid sequence duck polypeptide (SWISSPROT-ACC:P45882) ("P45882_Duck_DBI), and an 87amino acid human polypeptide (NZHU_Human_DBI). Regions with conservative amino acid substitutions are shown in gray. Non-conservative amino acid substitutions are presented without shading.

Table 27

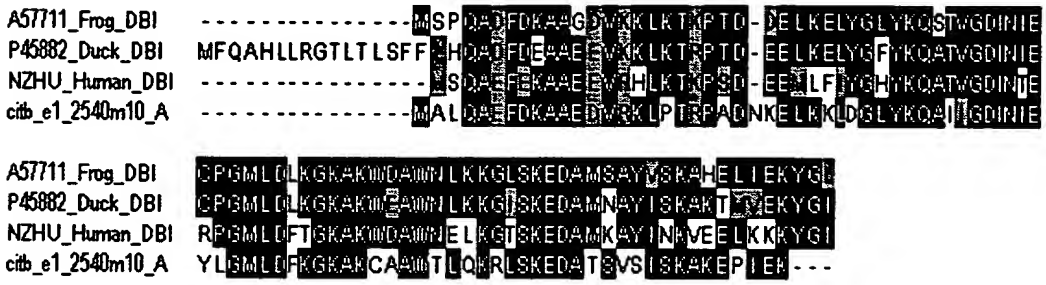


Table 27-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 27.

Table 27-1

SEQUENCE IDENTIFIER	SEQ ID NO
A57711_Frog_DBI	SEQ ID NO: 84
P45882_Duck_DBI	SEQ ID NO: 85
NZHU_Human_DBI	SEQ ID NO: 86
Citb_e1_2540m10_A	SEQ ID NO: 87

Using the PSORT program, it is predicted that the disclosed ENDO5 protein localizes to the cytoplasm with a certainty of 0.6500. In an analysis using the SIGNALP program, it is predicted that the ENDO5 protein does not possess a signal peptide.

The invention includes an ENDO5 nucleic acid encoding a polypeptide that includes the amino acid sequence of SEQ ID NO:10, *e.g.*, a nucleic acid including the nucleotide sequence of SEQ ID NO:9, or a fragment thereof. The invention also includes a mutant or variant nucleic acid, any one of whose bases may be changed from the corresponding base, as

illustrated in Table 22, while still encoding a protein which maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those previously described, including nucleic acid fragments that are complementary to any of the nucleic acids previously described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar-phosphate backbones are modified or derivatized. These modifications are carried out, at least in part, to enhance the chemical stability of the modified nucleic acid, such that they may be used, *e.g.*, as antisense binding nucleic acids in therapeutic applications.

An ENDO5 protein of the invention includes the polypeptide (SEQ ID NO:10) whose sequence is illustrated in Table 25. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue illustrated in Table 25, while still encoding a protein which maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptide

Metabolism-Regulating Peptide #7 (MRP-7) Sequence:

1 QAIIGDINIEYLGMLDFKGK (SEQ ID NO:21)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, which immunospecifically-bind to the ENDO5 polypeptide, and derivatives and fragments, thereof.

An ENDO5 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO5 is highly expressed in adipose and hematopietic tissue.

An ENDO5 sequence is also useful to modulate global energy metabolism or weight by altering serum cholesterol or glucose.

An ENDO5 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO5 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO6

An ENDO6 nucleic acid of the invention includes the nucleic acid sequence shown in Table 27A (SEQ ID NO:22).

Table 27A

1
ATGTTCCAGTTTCATGCAGGCTCTTGGGAAAGCTGGTGCTGCTGCTGCCTGATTCCCGCCGACAGACCTTGGGACCG
GGG
81
CCAACACTGGCAGCTGGAGATGGCGGACACGAGATCCGTGCACGAGACTAGGTTTGAGGCGGCCGTGAAGGTGATCC
AGA
161
GTTTGCCGAAGAATGGTTCATTCCAGCCAACAAATGAAATGATGCTTAAATTTTATAGCTTCTATAAGCAGGCAACT
GAA
241
GGACCCTGTAAACTTTCAAGGCCTGGATTTTGGGATCCTATTGGAAGATATAAATGGGATGCTTGGAGTTCACTGGG
TGA
321
TATGACCAAAGAGGAAGCCATGATTGCATATGTTGAAGAAATGAAAAAGATTATTGAAACTATGCCAATGACTGAGA
AAG
401
TTGAAGAATTGCTGCGTGTCATAGGTCCATTTTATGAAATTGTCGAGGACAAAAAGAGTGGCAGGAGTTCTGATATA
ACC
481
TCAGTCCGACTGGAGAAAATCTCTAAATGTTTAGAAGATCTTGGTAATGTTCTCACTTCTACTCCAAACGCCAAAAC
CGT
561
TAATGGTAAAGCTGAAAGCAGTGACAGTGGAGCGGAGTCTGAGGAAGAAGAGGCCCAAGAAGAAGTGAAAGGAGCAG
AAC
641
ACAGTGATAATGATAAGAAAATGATGAAGAAGTCAGCAGACCATAAGAATTTGGAAGTCATTGTCACTAATGGCTAT
GAT
721
AAAGATGGCTTTGTTCAGGATATACAGAATGACATTCATGCCAGTTCTTCCCTGAATGGCAGAAGCACTGAAGAAGT
AAA
801
GCCCATTGATGAAAACTTGGGGCAAACTGGAAAATCTGCTGTTTGCATTCACCAAGGTATTAATGATGATCATGTTG
AAG
881
ATGTTACAGGAATTCAGCATTTGACAAGCGATTCAGACAGTGAAGTTTACTGTGATTCTATGGAACAATTTGGACAA
GAA
961
GAGTCTTTAGACAGCTTTACGTCCAACAATGGACCATTTCAGTATTACTTGGGTGGTCATTCCAGTCAACCCATGGA
AAA
1041
TTCTGGATTTCGTGAAGATATTCAAGTACCTCCTGGAAATGGCAACATTGGGAATATGCAGGTGGTTGCAGTTGAAG
GAA
1121
AAGGTGAAGTCAAGCATGGAGGAGAAGATGGCAGGAATAACAGCGGAGCACCACACCGGGAGAAGCGAGGCGGAGAA
ACT

1201
GACGAATTCTCTAATGTTAGAAGAGGAAGAGGTCATAGGATGCAACACTTGAGCGAAGGAACCAAGGGCCGGCAGGT
GGG
1281
AAGTGGAGGTGATGGGGAGCGCTGGGGCTCCGACAGAGGGTCCCGAGGCAGCCTCAATGAGCAGATCGCCCTCGTGC
TGA
1361
TGAGACTGCAGGAGGACATGCAGAATGTCCTTCAGAGACTGCAGAAACTGGAAACGCTGACTGCTGCAAAATCATCA
ACA
1441
TCAACATTGCAGACTGCTCCTCAGCCCACCTCATCTCAGAGACCATCTTGGTGGCCCTTCGAGATGTCTCCTGGTGT
GCT
1521 AACGTTTGCCATCATATGGCCTTTTATTGCACAGTGGTTGGTGTATTTATACTATCAAAGAAGGAGAAGGTAA
(SEQ ID NO:22)

The nucleic acid sequence disclosed in Table 27A includes an open reading frame ("ORF") beginning at position 1 with start and stop codons indicated in bold. The ORF encodes a polypeptide sequence of 530 amino acid residues. The sequence of this encoded polypeptide (SEQ ID NO:23)is presented in Table 27B. The homology between the translated protein and Bovine endozepine (putativee ligand of benzodiazepine receptor) related protein (gb:GENBANK-ID:BOVEDZR|acc:M15888) is presented in Table 27C.

Table 27B

1
MFQFHAGSWESWCCCCLIPADRPWDRGQHWQLEMADTRSVHETRFEAAVKVIQSLPKNGSFQPTNEMMLKFYSFYKQ
ATE
81
GPCKLSRPGFWDPIGRYKWDAWSSLGDMTKEEAMIAYVEEMKKIIETMPMTEKVEELLRVIGPFYEIVEDKKSGRSS
DIT
161
SVRLEKISKCLEDLGNVLTSTPNAKTVNGKAESSDSGAESEEEEAQEEVKGAEHSDNDKKMMKKSADHKNLEVIVTN
GYD
241
KDGFVQDIQNDIHASSSLNGRSTEEVKPIDENLGQTGKSAVCIHQGINDDHVEDVTGIQHLTSDSDSEVYCDSMEQF
GQE
321
ESLDSFTSNNGPFQYYLGGHSSQPMENSGFREDIQVPPGNGNIGNMQVVAVEGKGEVKHGGEDGRNNSGAPHREKRG
GET
401
DEFSNVRRGRGHRMQHLSEGTKGRQVGSGGDGERWGSDRGSRGSLNEQIALVLMRLQEDMQNVLQRLQKLETLTAAK
SST
481 STLQTAPQPTSSQRPSWWPFEMSPGVLTFAIIWPFIAQWLVYLYYQRRRR (SEQ ID NO:23)

Translated Protein - Frame: 1 - Nucleotide 1 to 1590

Table 27C

>gb:GENBANK-ID:BOVEDZR|acc:M15888 Bovine endozepine (putativee ligand of benzodiazepine receptor) related protein mRNA, complete cds - Bos taurus, 1750 bp.

Length = 1750

Plus Strand HSPs:

Score = 2401 (845.2 bits), Expect = 2.9e-248, P = 2.9e-248
Identities = 448/530 (84%), Positives = 481/530 (90%), Frame = +2

```
Query:     1 MFQFHAGSWESWCCCC-LIPADRPWDRGQHWQLEMADTRSVHETRFEAAVKVIQSLPKNG 59
             |||||||||||||||| ||| |||||||+ |+|||  ||||||||||||||||||||||
Sbjct:    68 MFQFHAGSWESWCCCCCLIPGDRPWDRGRRWRLEMRHTRSVHETRFEAAVKVIQSLPKNG
247

Query:    60 SFQPTNEMMLKFYSFYKQATEGPCKLSRPGFWDPIGRYKWDAWSSLGDMTKEEAMIAYVE
119
             ||||||||||||||||||||||||||+||||||+||||||||||||||||||||||||||
Sbjct:   248 SFQPTNEMMLKFYSFYKQATEGPCKLSKPGFWDPVGRYKWDAWSSLGDMTKEEAMIAYVE
427

Query:   120 EMKKIIETMPMTEKVEELLRVIGPFYEIVEDKKSGRSSDITSVRLEKISKCLEDLGNVLT
179
             |||||+|||||||||||| ||||||||||||||||+||||||||||||||||||
Sbjct:   428 EMKKILETMPMTEKVEELLHVIGPFYEIVEDKKSGRSSDLTSVRLEKISKCLEDLGNVLA
607

Query:   180 STPNAKTVNGKAESSDSGAESEEEEAQEEVKGAEHSDNDKKMMKKSADHKNLEVIVTNGY
239
             |||||||||||||||||||||||| |||+ |  |  |+|||||||||||||+||||||
Sbjct:   608 STPNAKTVNGKAESSDSGAESEEEAAQEDPKRPEPRDSDKKMMKKSADHKNLEIIVTNGY
787

Query:   240 DKDGFVQDIQNDIHASSSLNGRSTEEVKPIDENLGQTGKSAVCIHQGINDDHVEDVTGIQ
299
             ||| ||| +|| || | ||||| ||||| +|||| ||||+ | +|| +| |||||++|||
Sbjct:   788 DKDSFVQGVQNSIHTSPSLNGRCTEEVKSVDENLEQTGKTVVFVHQDVNSDHVEDISGIQ
967

Query:   300 HLTSDSDSEVYCDSMEQFGQEESLDSFTSNNGPFQYYLGGHSSQPMENSGFREDIQVPPG
359
             |||||||||||||||||||||||| | |||||| |||||+ |||+|+|||  | +|  ||
Sbjct:   968 HLTSDSDSEVYCDSMEQFGQEESLDGFISNNGPFSYYLGGNPSQPLESSGFPEAVQGLPG
1147

Query:   360 NGNIGNMQVVAVEGKGEVKHGGEDGRNNSGAPHREKRGGETDEFSNVRRGRGHRMQHLSE
419
             ||+  +||   |||||||| ||||| +|||||||||| ||++||||+|||||||||||||
Sbjct:  1148 NGSPEDMQGAVVEGKGEVKRGGEDGGSNSGAPHREKRAGESEEFSNIRRGRGHRMQHLSE
1327

Query:   420 GTKGRQVGSGGDGERWGSDRGSRGSLNEQIALVLMRLQEDMQNVLQRLQKLETLTAAKSS
479
             |+|||||||||||||||||||||||||||||||||||||||||||||||||| ||| | |+++
Sbjct:  1328 GSKGRQVGSGGDGERWGSDRGSRGSLNEQIALVLMRLQEDMQNVLQRLHKLEMLAASQAK
1507

Query:   480 TSTLQTAPQPTSSQRPSWWPFEMSPGVLTFAIIWPFIAQWLVYLYYQRRRR 530 (SEQ ID
NO:88)
             +| |||+ ||||  |||||||||||| |||||||||||||+||||||||
Sbjct:  1508 SSALQTSNQPTSP-RPSWWPFEMSPGALTFAIIWPFIAQWLVHLYYQRRRR 1657 (SEQ
ID NO:89)
```

An ENDO6 nucleic acid of the invention can include a nucleic acid encoding the polypeptide of SEQ ID NO:23, *e.g.*, the ENDO6 nucleic acid can include the nucleic acid sequence of SEQ ID NO:22. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 27A. In some embodiments, the ENDO6 nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO6 polypeptide of the invention can include the amino acid sequence of SEQ ID NO:23. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:23, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof, such as the active peptide (SEQ ID NO:24)

Metabolism-Regulating Peptide #8 (MRP-8) Sequence:

QATEGPCKLSRPGFWDP (SEQ ID NO:24)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO6 polypeptide, and derivatives and fragments, thereof.

An ENDO6 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO6 is highly expressed in skeletal muscle. Also, high expression of ENDO6 is a marker for multiple types of cancer.

An ENDO6 sequence is also useful to modulate global energy metabolism or weight by altering serum cholesterol and insulin.

An ENDO6 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO6 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO7

An ENDO7 nucleic acid of the invention includes the nucleic acid sequence shown in Table 27D (SEQ ID NO:25).

Table 27D

```
1
       CCAGTATGTCTCAGGCGTTTGAGAAAGCTGCCAAGGATATTAAGCACCTTGAGACCAAGCCAGCAGATGATGAGAGG
ATG
81
       TTCATCTACAGCCGCTGCAAACAAGCGACTGTGCATGACTTAAATACAGAATGGCCCAGGATGTTAGACCTCAAAGG
CAA
161
       GGCAAAGCAGGATGCTTGGAATGAGCTGAAAGACACTGCCAAGGAAGATGCTGTGAAAGCTGATATCAACAAAGTAG
AAG
241    AGCGAAATAAAAAAATACAGAATATAAGAGATTG
(SEQ ID NO:25)
```

The nucleic acid sequence disclosed in Table 27D includes an open reading frame ("ORF") beginning at position 6 with start and stop codons indicated in bold. The ORF encodes a polypeptide sequence of 86 amino acid residues. The sequence of this encoded polypeptide is presented in Table 27E (SEQ ID NO:26). The homology between the translated protein and Bovine endozepine (putative ligand of benzodiazepine receptor) (gb:GENBANK-ID:BOVEDZ|acc:M15886) is presented in Table 27F.

Table 27E

```
1
       MSQAFEKAAKDIKHLETKPADDERMFIYSRCKQATVHDLNTEWPRMLDLKGKAKQDAWNELKDTAKEDAVKADINKV
EER
81     NKKYRI (SEQ ID NO:26)
```

Table 27F

```
>gb:GENBANK-ID:BOVEDZ|acc:M15886 Bovine endozepine (putative ligand of
benzodiazepine receptor) mRNA, complete cds - Bos taurus, 608 bp.
Length = 608

Plus Strand HSPs:

Score = 307 (108.1 bits), Expect = 6.5e-26, P = 6.5e-26
Identities = 62/87 (71%), Positives = 73/87 (83%), Frame = +2

Query:     1 MSQA-FEKAAKDIKHLETKPADDERMFIYSRCKQATVHDLNTEWPRMLDLKGKAKQDAWN 59
             |||| |+|||+++|||+|||||+| +|||| |||||  |+||| | ||| ||||| ||||
Sbjct:   125 MSQAEFDKAAEEVKHLKTKPADEEMLFIYSHYKQATVGDINTERPGMLDFKGKAKWDAWN
304

Query:    60 ELKDTAKEDAVKADINKVEERNKKYRI 86 (SEQ ID NO:90)
             ||| |+|||+|| |+||||  ||| |
Sbjct:   305 ELKGTSKEDAMKAYIDKVEELKKKYGI 385 (SEQ ID NO:91)
```

An ENDO7 nucleic acid of the invention can include a nucleic acid encoding the polypeptide of SEQ ID NO:26, *e.g.*, the ENDO7 nucleic acid can include the nucleic acid sequence of SEQ ID NO:25. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 27D. In some embodiments, the ENDO7 nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO7 polypeptide of the invention can include the amino acid sequence of SEQ ID NO:26. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:26, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptide (SEQ ID NO:27)

Metabolism-Regulating Peptide #9 (MRP-9) Sequence:

QATVHDLNTEWPRMLDLKGK (SEQ ID NO:27)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO7 polypeptide, and derivatives and fragments, thereof.

An ENDO7 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO7 is highly expressed in adipose tissue. Also, high expression of ENDO7 is a marker for liver cancer.

An ENDO7 sequence is also useful to modulate global energy metabolism or weight by altering serum cholesterol.

An ENDO7 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO7 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO8

An ENDO8 nucleic acid of the invention includes the nucleic acid sequence shown in Table 27G (SEQ ID NO:28).

Table 27G

1
ATGTGGGGCGACCTCTGGCTCCTCCCGCCTGCCTCTGCCAATCCGGGCACTGGGACAGAGGCTGAGTTTGAGAAAGC
TGC
81
AGAGGAGGTTAGGCACCTTAAGACCAAGCCATCGGATGAGGAGATGCTGTTCATCTATGGCCACTACAAACAAGCAA
CTG
161
TGGGCGACATAAATACAGAACGGCCCGGGATGTTGGACTTCACGGGCAAGGCCAAGTGGGATGCCTGGAATGAGCTG
AAA
241 *GGGACTTCCAAGGAAGATGCCATGAAAGCTTACATCAACAAAGTAGAAGAGCTAAAGAAAAAATACGGGATATGA*

(SEQ ID NO:28)

The nucleic acid sequence disclosed in Table 27G includes an open reading frame ("ORF") beginning at position 1 with start and stop codons in bold. The ORF encodes a

polypeptide sequence of 104 amino acid residues. The sequence of this encoded polypeptide is presented in Table 27H (SEQ ID NO:29). The homology between the translated protein and Human diazepam binding inhibitor (DBI) (gb:GENBANK-ID:HUMDBI|acc:M14200) is presented in Table 27I.

Table 27H

```
1
      MWGDLWLLPPASANPGTGTEAEFEKAAEEVRHLKTKPSDEEMLFIYGHYKQATVGDINTERPGMLDFTGKAKWDAWN
ELK
81    GTSKEDAMKAYINKVEELKKKYGI
(SEQ ID NO:29)
```

Table 27I

```
>gb:GENBANK-ID:HUMDBI|acc:M14200 Human diazepam binding inhibitor (DBI)
mRNA, complete cds - Homo sapiens, 556 bp.
Length = 556

Plus Strand HSPs:

Score = 562 (197.8 bits), Expect = 6.7e-53, P = 6.7e-53
Identities = 104/104 (100%), Positives = 104/104 (100%), Frame = +2

Query:     1 MWGDLWLLPPASANPGTGTEAEFEKAAEEVRHLKTKPSDEEMLFIYGHYKQATVGDINTE 60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:    20 MWGDLWLLPPASANPGTGTEAEFEKAAEEVRHLKTKPSDEEMLFIYGHYKQATVGDINTE
199

Query:    61 RPGMLDFTGKAKWDAWNELKGTSKEDAMKAYINKVEELKKKYGI 104 (SEQ ID NO:92)
             |||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   200 RPGMLDFTGKAKWDAWNELKGTSKEDAMKAYINKVEELKKKYGI 331 (SEQ ID NO:93)
```

An ENDO8 nucleic acid of the invention can include a nucleic acid encoding the polypeptide of SEQ ID NO:29, *e.g.*, the ENDO8 nucleic acid can include the nucleic acid sequence of SEQ ID NO:28. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 27G. In some embodiments, the ENDO8 nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to

enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO8 polypeptide of the invention can include the amino acid sequence of SEQ ID NO:29. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:29, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptide (SEQ ID NO:30)

Metabolism-Regulating Peptide #1 (MRP-1) Sequence:

1 QATVGDINTERPGMLDFTGK

(SEQ ID NO:30)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO8 polypeptide, and derivatives and fragments, thereof.

An ENDO8 sequence is useful for detecting specific types of tissue. For example when a panel of tissue is assayed for expression, ENDO8 is highly expressed in heart skeletal muscle, liver and endothelial tissue. Also, high expression of ENDO8 is a marker for breast and colon cancers as well as melanoma.

An ENDO8 sequence is also useful to modulate global energy metabolism or weight by altering serum cholesterol, insulin, or glucose. The ENDO8 sequence is also useful to modulate muscle mass or adipose level.

An ENDO8 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO8 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO9

An ENDO9 nucleic acid of the invention includes the nucleic acid sequence shown in Table 27J (SEQ ID NO:31).

Table 27J

1
ATGAGAGCCAGTCAGAAGGACTTTGAAAATTCAATGAATCAAGTGAAACTCTTGAAAAAGGATCCAGGAAACGAAGT
GAA
81
GCTAAAACTCTACGCGCTATATAAGCAGGCCACTGAAGGACCTTGTAACATGCCCAAACCAGGTGTATTTGACTTGA
TCA
161
ACAAGGCCAAATGGGACGCATGGAATGCCCTTGGCAGCCTGCCCAAGGAAGCTGCCAGGCAGAACTATGTGGATTTG
GTG
241
TCCAGTTTGAGTCCTTCATTGGAATCCTCTAGTCAGGTGGAGCCTGGAACAGACAGGAAATCAACTGGGTTTGAAAC
TCT
321
GGTGGTGACCTCCGAAGATGGCATCACAAAGATCATGTTCAACCGGCCCAAAAAGAAAAATGCCATAAACACTGAGA
TGT
401
ATCATGAAATTATGCGTGCACTTAAAGCTGCCAGCAAGGATGACTCAATCATCACTGTTTTAACAGGAAATGGTGAC
TAT
481
TACAGTAGTGGGAATGATCTGACTAACTTCACTGATATTCCCCCTGGTGGAGTAGAGGAGAAAGCTAAAAATAATGC
CGT
561
TTTACTGAGGGAATTTGTGGGCTGTTTTATAGATTTTCCTAAGCCTCTGATTGCAGTGGTCAATGGTCCAGCTGTGG
GCA
641
TCTCCGTCACCCTCCTTGGGCTATTCGATGCCGTGTATGCATCTGACAGGGCAACATTTCATACACCATTTAGTCAC
CTA
721
GGCCAAAGTCCGGAAGGATGCTCCTCTTACACTTTTCCGAAGATAATGAGCCCAGCCAAGGCAACAGAGATGCTTAT
TTT
801
TGGAAAGAAGTTAACAGCGGGAGAGGCATGTGCTCAAGGACTTGTTACTGAAGTTTTCCCTGATAGCACTTTTCAGA
AAG
881
AAGTCTGGACCAGGCTGAAGGCATTTGCAAAGCTTCCCCCAAATGCCTTGAGAATTTCAAAAGAGGTAATCAGGAAA
AGA
961
GAGAGAGAAAAACTACACGCTGTTAATGCTGAAGAATGCAATGTCCTTCAGGGAAGATGGCTATCAGATGAATGCAC
AAA
1041 *TGCTGTGGTGAACTTCTTATCCAGAAAATCAAAACTGTGA*

(SEQ ID NO:31)

The nucleic acid sequence disclosed in Table 27J includes an open reading frame ("ORF") beginning at position 1 with start and stop codons in bold. The ORF encodes a polypeptide sequence of 359 amino acid residues. The sequence of this encoded polypeptide is presented in Table 27K (SEQ ID NO:32). The homology between the translated protein and Homo sapiens peroxisomal D3,D2-enoyl-CoA isomerase (PECI) (gb:GENBANK-ID:AF153612|acc:AF153612) is presented in Table 27L.

Table 27K

1
MRASQKDFENSMNQVKLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNALGSLPKEAARQNYV
DLV
81
SSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGITKIMFNRPKKKNAINTEMYHEIMRALKAASKDDSIITVLTGN
GDY
161
YSSGNDLTNFTDIPPGGVEEKAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVGISVTLLGLFDAVYASDRATFHTPF
SHL
241
GQSPEGCSSYTFPKIMSPAKATEMLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNALRISKEVI
RKR
321 EREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL
(SEQ ID NO:32)

Table 27L

```
>gb:GENBANK-ID:AF153612|acc:AF153612 Homo sapiens peroxisomal
D3,D2-enoyl-CoA isomerase (PECI) mRNA, complete cds - Homo
sapiens, 1348 bp.
Length = 1348
Plus Strand HSPs:

Score = 1857 (653.7 bits), Expect = 1.6e-190, P = 1.6e-190
Identities = 359/359 (100%), Positives = 359/359 (100%), Frame = +1

Query:     1 MRASQKDFENSMNQVKLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDA 60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   103 MRASQKDFENSMNQVKLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDA
282

Query:    61 WNALGSLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGITKIMF
120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   283 WNALGSLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGITKIMF
462

Query:   121 NRPKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSSGNDLTNFTDIPPGGVEE
180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   463 NRPKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSSGNDLTNFTDIPPGGVEE
642

Query:   181 KAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVGISVTLLGLFDAVYASDRATFHTPFSHL
240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   643 KAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVGISVTLLGLFDAVYASDRATFHTPFSHL
822

Query:   241 GQSPEGCSSYTFPKIMSPAKATEMLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLK
300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   823 GQSPEGCSSYTFPKIMSPAKATEMLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLK
1002

Query:   301 AFAKLPPNALRISKEVIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL 359
(SEQ ID NO:94)
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:  1003 AFAKLPPNALRISKEVIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL
1179 (SEQ ID NO:95)
```

An ENDO9 nucleic acid of the invention can include a nucleic acid encoding the polypeptide of SEQ ID NO:32, *e.g.*, the ENDO9 nucleic acid can include the nucleic acid sequence of SEQ ID NO:31. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 27J. In some embodiments, the ENDO9 nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just

described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO9 polypeptide of the invention can include the amino acid sequence of SEQ ID NO:32. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:32, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptide (SEQ ID NO:33)

Metabolism-Regulating Peptide #2 (MRP-2) Sequence:

QATEGPCNMPKPGVFDLINK

(SEQ ID NO:33)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO9 polypeptide, and derivatives and fragments, thereof.

An ENDO9 sequence is also useful to modulate global energy metabolism or weight by altering serum cholesterol, insulin, or glucose.

An ENDO9 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO9 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDO10

An ENDO10 nucleic acid of the invention includes the nucleic acid sequence shown in Table 27M (SEQ ID NO:34).

Table 27M

1
TCCTTCCCCCACCCCCGGGGGCCCATCCCGGTGGCGGGCTCCGGAGCTCGGGACTGCTAATTTCAGCGAAACGATTA
AAA
81
GACGCCCCTACAGCTGACGGCACTTTCTCTCCTCCGGCAGGANAGGACGTCCAGCGTACGTCNGCCCGCGCTTCCCC
GCC
161
GGCGCAGAGCAGGCCTCACAGAATCGCACGCCGCTGGCACGCACGCCGCCCCGCCCCCACGGCCCAGCGCCAGCGCG
CCC
241
CGCGTCGCACGCATCCCGGCCTCACTGCCCCTCGACTCCTGTTCCGTTGGAGGGGCCTGAGGCGAGCCTGAGCGCGC
TGT
321
TGGCCGGAGGAAGCCGGAGAGACCGGGTCGACTGGGCAGAGCGGCAGAGGGTCGAGGAGCCTGCTCTGCACGCCCAG
GGA
401
GTAGAAGTGGGCAGGGAGCAGGGTCACGTGAGGGAGCGCGCCGCGACTGAGCTTGGGTCCGACTGGAGCTCAGGCTC
GCG
481
ACCCAGACTGGTGGGCCAGGCCTCCAAGCCGGCCTTACACCCAATCCAAGGAGGACAGACCGGACACAGAGGGACGG
AGC
561
GAGCAAGGAGACATGGCTTCATCATTCCTGCCCGCGGGGGCCATCACCGGCGACAGCGGTGGAGAGCTGAGCTCAGG
GGA
641
CGACTCCGGGGAGGTGGAGTTCCCCCATAGCCCTGAGATCGAGGAGACCAGTTGCCTGGCCGAGCTGTTTGAGAAGG
CTG
721
CCGCGCACCTGCAAGGCCTGATTCAGGTGGCCAGCAGGGAGCAGCTCTTGTACCTGTATGCCAGGTACAAACAGGTC
AAA
801
GTTGGAAATTGTAATACTCCTAAACCAAGCTTCTTTGATTTTGAAGGAAAGCAAAAATGGGAAGCTTGGAAAGCACT
TGG
881
TGATTCAAGCCCCAGCCAAGCAATGCAGGAATATATCGCAGTAGTTAAAAAACTAGATCCAGGTTGGAATCCTCAGA
TAC
961
CAGAGAAGAAAGGAAAAGAAGCAAATACAGGTTTTGGTGGGCCAGTTATTAGTTCTCTATATCATGAAGAAACCATC
AGG
1041
GAAGAAGACAAAAATATATTTGATTACTGCAGGGAAAACAACATTGACCATATAACCAAAGCCATCAAATCGAAAAA
TGT
1121
GGATGTGAATGTGAAAGATGAAGAGGGTAGGGCTCTACTTCACTGGGCCTGTGATCGAGGACATAAGGAACTAGTCA
CAG
1201
TGTTGCTGCAACATAGAGCTGACATTAACTGTCAGGACAATGAAGGCCAAACAGCTCTACATTATGCCTCTGCCTGT
GAG
1281
TTTCTGGATATTGTAGAGCTGCTGCTCCAGTCTGGTGCTGACCCCACTCTCCGAGACCAGGATGGCTGCCTGCCAGA
GGA
1361
GGTGACAGGCTGCAAAACAGTTTCTTTGGTGCTGCAGCGGCACACAACTGGCAAGGCTTAATCAAAAGACTGGAAAA
CTG
1441
CAGTCTGTAATAGCATAAGGCTTCCATTATGAAAGAAAACTACAAAAATAATACTTCTTTTCCACCCGTCTTTGGTA
TGT

1521 ATTGGCTAATAAAATCAGTTCTGTGGAACTGGGAAAAAAAAAAAAAAAAAAAAA

(SEQ ID NO:34)

The nucleic acid sequence disclosed in Table 27M includes an open reading frame ("ORF") beginning at position 573 with start and stop codons in bold. The ORF encodes a polypeptide sequence of 282 amino acid residues. The sequence of this encoded polypeptide is presented in Table 27N (SEQ ID NO:35). The homology between the translated protein and(Human DBI/ACBP-like protein Patent: US 5734038-A 2 31-MAR-1998) is presented in Table 27O.

Table 27N

```
1
      MASSFLPAGAITGDSGGELSSGDDSGEVEFPHSPEIEETSCLAELFEKAAAHLQGLIQVASREQLLYLYARYKQVKV
GNC
81
      NTPKPSFFDFEGKQKWEAWKALGDSSPSQAMQEYIAVVKKLDPGWNPQIPEKKGKEANTGFGGPVISSLYHEETIRE
EDK
161
      NIFDYCRENNIDHITKAIKSKNVDVNVKDEEGRALLHWACDRGHKELVTVLLQHRADINCQDNEGQTALHYASACEF
LDI
241   VELLLQSGADPTLRDQDGCLPEEVTGCKTVSLVLQRHTTGKA
(SEQ ID NO:35)
```

Table 27O

```
>gb:GENBANK-ID:I96163|acc:I96163 Sequence 2 from patent US 5734038 -
Unknown., 1123 bp.
Length = 1123
Plus Strand HSPs:

Score = 1340 (471.7 bits), Expect = 1.2e-135, P = 1.2e-135
Identities = 259/282 (91%), Positives = 262/282 (92%), Frame = +1

Query:     1 MASSFLPAGAITGDSGGELSSGDDSGEVEFPHSPEIEETSCLAELFEKAAAHLQGLIQVA 60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   121 MASSFLPAGAITGDSGGELSSGDDSGEVEFPHSPEIEETSCLAELFEKAAAHLQGLIQVA
300

Query:    61 SREQLLYLYARYKQVKVGNCNTPKPSFFDFEGKQKWEAWKALGDSSPSQAMQEYIAVVKK
120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   301 SREQLLYLYARYKQVKVGNCNTPKPSFFDFEGKQKWEAWKALGDSSPSQAMQEYIAVVKK
480

Query:   121 LDPGWNPQIPEKKGKEANTGFGGPVISSLYHEETIREEDKNIFDYCRENNIDHITKAIKS
180
             |||||||||||||  | +          |+      +  ||||||||||||||||||||
Sbjct:   481 LDPGWNPQIPEKKRKRSKYKVWASY*FSIS*RNH-QGRDKNIFDYCRENNIDHITKAIKS
657
```

```
Query:   181 KNVDVNVKDEEGRALLHWACDRGHKELVTVLLQHRADINCQDNEGQTALHYASACEFLDI
240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   658 KNVDVNVKDEEGRALLHWACDRGHKELVTVLLQHRADINCQDNEGQTALHYASACEFLDI
837

Query:   241 VELLLQSGADPTLRDQDGCLPEEVTGCKTVSLVLQRHTTGKA 282 (SEQ ID NO:96)
             ||||||||||||||||||||||||||||||||||||||||||
Sbjct:   838 VELLLQSGADPTLRDQDGCLPEEVTGCKTVSLVLQRHTTGKA 963 (SEQ ID NO:97)
```

An ENDO10 nucleic acid of the invention can include a nucleic acid encoding the polypeptide of SEQ ID NO:35, *e.g.*, the ENDO10 nucleic acid can include the nucleic acid sequence of SEQ ID NO:34. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 27M. In some embodiments, the ENDO10 nucleic acid encodes a protein that maintains its endozepine-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, but are not limited to: modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

An ENDO10 polypeptide of the invention can include the amino acid sequence of SEQ ID NO:35. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:35, while still encoding a protein that maintains its endozepine-like activities and physiological functions, or a functional fragment thereof such as the following active peptide (SEQ ID NO:36)

Metabolism-Regulating Peptide #10 (MRP-10) Sequence:

1 QVKVGNCNTPKPSFFDFEGK

(SEQ ID NO:36)

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to the ENDO10 polypeptide, and derivatives and fragments, thereof.

An ENDO10 sequence is also useful to modulate global energy metabolism or weight by altering serum insulin or glucose. The ENDO10 sequence is also useful to modulate muscle mass or adipose level

An ENDO10 sequence is also useful in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art.

An ENDO10 sequence is useful in the treatment of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers by modulating metabolism.

ENDOX nucleic acids encode polypeptides that are novel members of the endozepine family. The endozepines have been reported to be involved in multiple biologically importatnt functions. Related endozepine polypeptides, such as diazepam binding inhibitor, bind GABA receptors. Accordingly, the new ENDOX polypeptides, or fragments or variants thereof, can be used in, *e.g.*, screening assays to identify new agonists or antagonists for GABA receptors. The new ENDOX polypeptides and also be used to modulate GABA receptor activity.

ENDOX polypeptides, nucleic acids, antibodies, and other compositions according to the invention also have utilities based on other known functions of endozepine family members. For example, diazepam binding inhibitor, is also known as acyl-CoA binding protein (ACBP). Acyl-CoA-binding protein binds to medium- and long-chain acyl-CoA esters with high affinity, and may act as an intra-cellular carrier of acyl-CoA esters. Thus,

The ACBP gene has also been cloned in yeast. The yeast cognate is named acyl-CoA binding (ACB) (Rose et al., Proc. Nat. Acad. Sci. (USA) 89: 11287-11291). The yeast gene encodes a polypeptide of 87 amino acid residues (including the initiating methionine), which is identical in length to the human gene product. The yeast polypeptide is 48% conserved with human amino acid residues. The most highly conserved yeast domain was found to comprise a total of 7 contiguous amino acid residues which are identical in all known protein species from yeast, birds, and mammals. This domain constitutes the hydrophobic binding site for acyl-CoA esters, and is located within the second helical region of the molecule. The

presence of such a highly conserved gene in primitive organisms (*e.g.*, yeast) supports its basic biological role as an acyl-CoA binding protein and also suggests that many of the biological functions attributed to it in higher organisms may result from its ability to interact with acyl-CoA.

Various endozepine family members, or derivatives of these polypeptides, have also been identified as antibacterial peptides. Examples include cecropin P1 and PR-39. PR-39 is a 39 amino acid residue proline- and arginine-rich polypeptide isolated from the upper part of pig small intestine. Amino acid sequence analysis in combination with mass spectrometry identified two of three of the the peptides as gastric inhibitory polypeptide (7-42) (GIP(7-42)) and diazepam-binding inhibitor (32-86) (DBI(32-86)), derived from factors which had been previously identified. The third polypeptide constituted a previously-unknown structure, which was designated peptide 3910, in relation to its molecular mass. All three polypeptides demonstrate antibacterial activity against *Bacillus megaterium*. GIP (7-42) also shows some activity against *Streptococcus pyogenes* and an *Escherichia coli* mutant with a defect in its outer membrane.

A summary of the ENDOX nucleic acid sequences, encoded ENDOX polypeptides, as well as Sequence Identifier Numbers (SEQ ID NOS) corresponding to various disclosed sequences and clones containing these nucleic acids, is shown in Table 28, below.

Table 28: Disclosed Sequences and Corresponding SEQ ID Numbers of ENDOX Polypeptides

ENDOX	Sequence Identifier Number of Disclosed Nucleic Acid Sequence	ORF start and stop codons from corresponding nucleic acid	Sequence Identifier Number of Encoded Polypeptide Sequence	Sequence Identifier Number of Active Peptide
ENDO1	SEQ ID NO:1 SEQ ID NO: 46 SEQ ID NO:48	1-267 1-687 1-576	SEQ ID NO:2 SEQ ID NO:47 SEQ ID NO:49	SEQ ID NO:15
ENDO2	SEQ ID NO:3	58-321	SEQ ID NO:4	SEQ ID NO:16
ENDO3	SEQ ID NO:5	83-496	SEQ ID NO:6	SEQ ID NO:17,18
ENDO4	SEQ ID NO:7	11-298	SEQ ID NO:8	SEQ ID NO:19,20
ENDO5	SEQ ID NO:9	7-264	SEQ ID NO:10	SEQ ID NO:21
ENDO6	SEQ ID NO:22	1-1590	SEQ ID NO:23	SEQ ID NO:24
ENDO7	SEQ ID NO:25	6-263	SEQ ID NO:26	SEQ ID NO:27
ENDO8	SEQ ID NO:28	1-312	SEQ ID NO:29	SEQ ID NO:30
ENDO9	SEQ ID NO:31	1-1077	SEQ ID NO:32	SEQ ID NO:33
ENDO10	SEQ ID NO:34	573-1418	SEQ ID NO:35	SEQ ID NO:36

ENDOX Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode ENDOX polypeptides or biologically-active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify ENDOX-encoding nucleic acids (*e.g.*, ENDOX mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of ENDOX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An ENDOX nucleic acid can encode a mature ENDOX polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The

product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated ENDOX nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb,

0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can

be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48 as a hybridization probe, ENDOX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to ENDOX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or a portion of this nucleotide sequence (*e.g.*, a

fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an ENDOX polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95%

identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of ENDOX polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an ENDOX polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human ENDOX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, as well as a polypeptide possessing ENDOX biological activity. Various biological activities of the ENDOX proteins are described below.

An ENDOX polypeptide is encoded by the open reading frame ("ORF") of an ENDOX nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human ENDOX genes allows for the generation of probes and primers designed for use in identifying and/or cloning ENDOX homologues in other cell types, *e.g.* from other tissues, as well as ENDOX homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48; or an anti-sense strand nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48; or of a naturally occurring mutant of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48.

Probes based on the human ENDOX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an ENDOX protein, such as by measuring a level of an ENDOX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting ENDOX mRNA levels or determining whether a genomic ENDOX gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an ENDOX polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of ENDOX" can be prepared by isolating a portion of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, that encodes a polypeptide having an ENDOX biological activity (the biological activities of the ENDOX proteins are described below), expressing the encoded portion of ENDOX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of ENDOX.

ENDOX Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, due to degeneracy of the genetic code and thus encode the same ENDOX proteins as that encoded by the nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide

sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49.

In addition to the human ENDOX nucleotide sequences shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the ENDOX polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the ENDOX genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an ENDOX protein, preferably a vertebrate ENDOX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the ENDOX genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the ENDOX polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the ENDOX polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding ENDOX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the ENDOX cDNAs of the invention can be isolated based on their homology to the human ENDOX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding ENDOX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or

high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31,

34, 46, and 48, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel ,*et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* **78**: 6789-6792.

Conservative Mutations

In addition to naturally-occurring allelic variants of ENDOX sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, thereby leading to changes in the amino acid sequences of the encoded ENDOX proteins, without altering the functional ability of said ENDOX proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the ENDOX proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the ENDOX

proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding ENDOX proteins that contain changes in amino acid residues that are not essential for activity. Such ENDOX proteins differ in amino acid sequence from SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49; more preferably at least about 70% homologous to SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49; still more preferably at least about 80% homologous to SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49; even more preferably at least about 90% homologous to SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49; and most preferably at least about 95% homologous to SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49.

An isolated nucleic acid molecule encoding an ENDOX protein homologous to the protein of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with

basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the ENDOX protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an ENDOX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for ENDOX biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant ENDOX protein can be assayed for (*i*) the ability to form protein:protein interactions with other ENDOX proteins, other cell-surface proteins, or biologically-active portions thereof, (*ii*) complex formation between a mutant ENDOX protein and an ENDOX ligand; or (*iii*) the ability of a mutant ENDOX protein to bind to an intracellular target protein or biologically-active portion thereof; (*e.g.* avidin proteins).

In yet another embodiment, a mutant ENDOX protein can be assayed for the ability to regulate a specific biological function (*e.g.*, regulation of insulin release).

Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (*e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire ENDOX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an ENDOX protein of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49; or

antisense nucleic acids complementary to an ENDOX nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an ENDOX protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the ENDOX protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the ENDOX protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of ENDOX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of ENDOX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of ENDOX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil,

queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an ENDOX protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other. See, *e.g.*, Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, *e.g.*, Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, *e.g.*, Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330.

Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These

modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave ENDOX mRNA transcripts to thereby inhibit translation of ENDOX mRNA. A ribozyme having specificity for an ENDOX-encoding nucleic acid can be designed based upon the nucleotide sequence of an ENDOX cDNA disclosed herein (*i.e.*, SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an ENDOX-encoding mRNA. See, *e.g.*, U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* ENDOX mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, ENDOX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the ENDOX nucleic acid (*e.g.*, the ENDOX promoter and/or enhancers) to form triple helical structures that prevent transcription of the ENDOX gene in target cells. *See, e.g.,* Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the ENDOX nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. *See, e.g.,* Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.*, DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of ENDOX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of ENDOX can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S_1 nucleases (*see,* Hyrup, *et al.,* 1996.*supra*); or as probes or primers for DNA sequence and hybridization (*see,* Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.,* 1996. *supra*).

In another embodiment, PNAs of ENDOX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of ENDOX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see,* Hyrup, etal., 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.,* 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.,* Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.,* Finn, *et al.*, 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.,* Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, *et al.*, 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.,* Krol, *et al.*, 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon, 1988.

Pharm. Res. 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

ENDOX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of ENDOX polypeptides whose sequences are provided in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, while still encoding a protein that maintains its ENDOX activities and physiological functions, or a functional fragment thereof.

In general, an ENDOX variant that preserves ENDOX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated ENDOX proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-ENDOX antibodies. In one embodiment, native ENDOX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, ENDOX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an ENDOX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the ENDOX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of ENDOX proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes

preparations of ENDOX proteins having less than about 30% (by dry weight) of non-ENDOX proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-ENDOX proteins, still more preferably less than about 10% of non-ENDOX proteins, and most preferably less than about 5% of non-ENDOX proteins. When the ENDOX protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the ENDOX protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of ENDOX proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of ENDOX proteins having less than about 30% (by dry weight) of chemical precursors or non-ENDOX chemicals, more preferably less than about 20% chemical precursors or non-ENDOX chemicals, still more preferably less than about 10% chemical precursors or non-ENDOX chemicals, and most preferably less than about 5% chemical precursors or non-ENDOX chemicals.

Biologically-active portions of ENDOX proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the ENDOX proteins (*e.g.*, the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49) that include fewer amino acids than the full-length ENDOX proteins, and exhibit at least one activity of an ENDOX protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the ENDOX protein. A biologically-active portion of an ENDOX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native ENDOX protein.

In an embodiment, the ENDOX protein has an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49. In other embodiments, the ENDOX protein is substantially homologous to SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45

inclusive, 47, and 49, and retains the functional activity of the protein of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the ENDOX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49 and retains the functional activity of the ENDOX proteins of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49.

Determining Homology Between Two or More Sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See,* Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of

nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

Chimeric and Fusion Proteins

The invention also provides ENDOX chimeric or fusion proteins. As used herein, an ENDOX "chimeric protein" or "fusion protein" comprises an ENDOX polypeptide operatively-linked to a non-ENDOX polypeptide. An "ENDOX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an ENDOX protein (SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49), whereas a "non-ENDOX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the ENDOX protein, *e.g.*, a protein that is different from the ENDOX protein and that is derived from the same or a different organism. Within an ENDOX fusion protein the ENDOX polypeptide can correspond to all or a portion of an ENDOX protein. In one embodiment, an ENDOX fusion protein comprises at least one biologically-active portion of an ENDOX protein. In another embodiment, an ENDOX fusion protein comprises at least two biologically-active portions of an ENDOX protein. In yet another embodiment, an ENDOX fusion protein comprises at least three biologically-active portions of an ENDOX protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the ENDOX polypeptide and the non-ENDOX polypeptide are fused in-frame with one another. The non-ENDOX polypeptide can be fused to the N-terminus or C-terminus of the ENDOX polypeptide.

In one embodiment, the fusion protein is a GST-ENDOX fusion protein in which the ENDOX sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant ENDOX polypeptides.

In another embodiment, the fusion protein is an ENDOX protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host

cells), expression and/or secretion of ENDOX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an ENDOX-immunoglobulin fusion protein in which the ENDOX sequences are fused to sequences derived from a member of the immunoglobulin protein family. The ENDOX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an ENDOX ligand and an ENDOX protein on the surface of a cell, to thereby suppress ENDOX-mediated signal transduction *in vivo*. The ENDOX-immunoglobulin fusion proteins can be used to affect the bioavailability of an ENDOX cognate ligand. Inhibition of the ENDOX ligand/ENDOX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the ENDOX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-ENDOX antibodies in a subject, to purify ENDOX ligands, and in screening assays to identify molecules that inhibit the interaction of ENDOX with an ENDOX ligand.

An ENDOX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.*, Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An ENDOX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the ENDOX protein.

ENDOX Agonists and Antagonists

The invention also pertains to variants of the ENDOX proteins that function as either ENDOX agonists (*i.e.*, mimetics) or as ENDOX antagonists. Variants of the ENDOX protein

can be generated by mutagenesis (*e.g.*, discrete point mutation or truncation of the ENDOX protein). An agonist of the ENDOX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the ENDOX protein. An antagonist of the ENDOX protein can inhibit one or more of the activities of the naturally occurring form of the ENDOX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the ENDOX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the ENDOX proteins.

Variants of the ENDOX proteins that function as either ENDOX agonists (*i.e.*, mimetics) or as ENDOX antagonists can be identified by screening combinatorial libraries of mutants (*e.g.*, truncation mutants) of the ENDOX proteins for ENDOX protein agonist or antagonist activity. In one embodiment, a variegated library of ENDOX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of ENDOX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential ENDOX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of ENDOX sequences therein. There are a variety of methods which can be used to produce libraries of potential ENDOX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential ENDOX sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.*, Narang, 1983. *Tetrahedron* 39: 3; Itakura, *et al.*, 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.*, 1984. *Science* 198: 1056; Ike, *et al.*, 1983. *Nucl. Acids Res.* 11: 477.

Polypeptide Libraries

In addition, libraries of fragments of the ENDOX protein coding sequences can be used to generate a variegated population of ENDOX fragments for screening and subsequent selection of variants of an ENDOX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an ENDOX coding

sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S_1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the ENDOX proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of ENDOX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify ENDOX variants. *See, e.g.,* Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

Anti-ENDOX Antibodies

The invention encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the ENDOX polypeptides of said invention.

An isolated ENDOX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind to ENDOX polypeptides using standard techniques for polyclonal and monoclonal antibody preparation. The full-length ENDOX proteins can be used or, alternatively, the invention provides antigenic peptide fragments of ENDOX proteins for use as immunogens. The antigenic ENDOX peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, and encompasses an epitope of ENDOX such that an antibody raised against the peptide forms a specific immune complex with ENDOX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes

preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of ENDOX that is located on the surface of the protein (*e.g.*, a hydrophilic region). As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (*see, e.g.*, Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety).

As disclosed herein, ENDOX protein sequences of SEQ ID NO:2, 4, 6, 8, 10, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as ENDOX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human ENDOX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an ENDOX protein sequence of SEQ ID NO:2, 4, 6, 8, 10, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed ENDOX protein or a chemically-synthesized ENDOX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against ENDOX can be isolated from the mammal (*e.g.*, from the blood)

and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of ENDOX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular ENDOX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular ENDOX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (*see, e.g.,* Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*see, e.g.,* Kozbor, *et al.,* 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*see, e.g.,* Cole, *et al.,* 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (*see, e.g.,* Cote, *et al.,* 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see, e.g.,* Cole, *et al.,* 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an ENDOX protein (*see, e.g.,* U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (*see, e.g.,* Huse, *et al.,* 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for an ENDOX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. *See, e.g.,* U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to an ENDOX protein may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (*iv*) F_v fragments.

Additionally, recombinant anti-ENDOX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made

using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an ENDOX protein is facilitated by generation of hybridomas that bind to the fragment of an ENDOX protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an ENDOX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-ENDOX antibodies may be used in methods known within the art relating to the localization and/or quantitation of an ENDOX protein (*e.g.,* for use in measuring levels of the ENDOX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for ENDOX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-ENDOX antibody (*e.g.*, monoclonal antibody) can be used to isolate an ENDOX polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-ENDOX antibody can facilitate the purification of natural ENDOX polypeptide from cells and of recombinantly-produced ENDOX polypeptide

expressed in host cells. Moreover, an anti-ENDOX antibody can be used to detect ENDOX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the ENDOX protein. Anti-ENDOX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

ENDOX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an ENDOX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, ENDOX proteins, mutant forms of ENDOX proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of ENDOX proteins in prokaryotic or eukaryotic cells. For example, ENDOX proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to

increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.,* Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.,* Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the ENDOX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, ENDOX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.,* SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors

include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α-fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to ENDOX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene

expression using antisense genes *see, e.g.,* Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, ENDOX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding ENDOX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) ENDOX protein. Accordingly, the invention further provides methods for producing ENDOX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding ENDOX protein has been introduced) in a suitable medium such that ENDOX protein is produced. In another embodiment, the method further comprises isolating ENDOX protein from the medium or the host cell.

Transgenic ENDOX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which ENDOX protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous ENDOX sequences have been introduced into their genome or homologous recombinant animals in which endogenous ENDOX sequences have been altered. Such animals are useful for studying the function and/or activity of ENDOX protein and for identifying and/or evaluating modulators of ENDOX protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous ENDOX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing ENDOX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human ENDOX cDNA sequences of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human ENDOX gene, such as a mouse ENDOX gene, can be

isolated based on hybridization to the human ENDOX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the ENDOX transgene to direct expression of ENDOX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the ENDOX transgene in its genome and/or expression of ENDOX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding ENDOX protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an ENDOX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the ENDOX gene. The ENDOX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48), but more preferably, is a non-human homologue of a human ENDOX gene. For example, a mouse homologue of human ENDOX gene of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, can be used to construct a homologous recombination vector suitable for altering an endogenous ENDOX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous ENDOX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous ENDOX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous ENDOX protein). In the homologous recombination vector, the altered portion of the ENDOX gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the ENDOX gene to allow for homologous recombination to occur between the exogenous ENDOX gene carried by the vector and an endogenous ENDOX gene in an embryonic stem cell. The additional flanking ENDOX nucleic acid is of sufficient length for successful

homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.,* Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is ten introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced ENDOX gene has homologously-recombined with the endogenous ENDOX gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae. See,* O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to

morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.*, the somatic cell) is isolated.

Pharmaceutical Compositions

The ENDOX nucleic acid molecules, ENDOX proteins, and anti-ENDOX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an ENDOX protein or anti-ENDOX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or

adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used

herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.,* U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express ENDOX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect ENDOX mRNA (*e.g.*, in a biological sample) or a genetic lesion in an ENDOX gene, and to modulate ENDOX activity, as described further, below. In addition, the ENDOX proteins can be used to screen drugs or compounds that modulate the ENDOX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of ENDOX protein or production of ENDOX protein forms that have decreased or aberrant activity compared to ENDOX wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease(possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-ENDOX antibodies of the invention can be used to detect and isolate ENDOX proteins and modulate ENDOX activity. In yet a further aspect,

the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to ENDOX proteins or have a stimulatory or inhibitory effect on, *e.g.*, ENDOX protein expression or ENDOX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an ENDOX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.,* Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of ENDOX protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an ENDOX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the ENDOX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the ENDOX protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of ENDOX protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds ENDOX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an ENDOX protein, wherein determining the ability of the test compound to interact with an ENDOX protein comprises determining the ability of the test compound to preferentially bind to ENDOX protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of ENDOX protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the ENDOX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of ENDOX or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the ENDOX protein to bind to or interact with an

ENDOX target molecule. As used herein, a "target molecule" is a molecule with which an ENDOX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an ENDOX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An ENDOX target molecule can be a non-ENDOX molecule or an ENDOX protein or polypeptide of the invention . In one embodiment, an ENDOX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound ENDOX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with ENDOX.

Determining the ability of the ENDOX protein to bind to or interact with an ENDOX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the ENDOX protein to bind to or interact with an ENDOX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an ENDOX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an ENDOX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the ENDOX protein or biologically-active portion thereof. Binding of the test compound to the ENDOX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the ENDOX protein or biologically-active portion thereof with a known compound which binds ENDOX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an ENDOX protein, wherein determining the ability of the test compound to interact with an ENDOX protein comprises determining the ability of the test compound to preferentially bind to ENDOX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting ENDOX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the ENDOX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of ENDOX can be accomplished, for example, by determining the ability of the ENDOX protein to bind to an ENDOX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of ENDOX protein can be accomplished by determining the ability of the ENDOX protein further modulate an ENDOX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the ENDOX protein or biologically-active portion thereof with a known compound which binds ENDOX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an ENDOX protein, wherein determining the ability of the test compound to interact with an ENDOX protein comprises determining the ability of the ENDOX protein to preferentially bind to or modulate the activity of an ENDOX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of ENDOX protein. In the case of cell-free assays comprising the membrane-bound form of ENDOX protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of ENDOX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either ENDOX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to ENDOX protein, or interaction of ENDOX protein with a target molecule in the presence and absence of a candidate compound,

can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-ENDOX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or ENDOX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of ENDOX protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the ENDOX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated ENDOX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with ENDOX protein or target molecules, but which do not interfere with binding of the ENDOX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or ENDOX protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the ENDOX protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the ENDOX protein or target molecule.

In another embodiment, modulators of ENDOX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of ENDOX mRNA or protein in the cell is determined. The level of expression of ENDOX mRNA or protein in the presence of the candidate compound is compared to the level of expression of ENDOX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of ENDOX mRNA or protein expression based upon this comparison. For example, when expression of ENDOX mRNA or protein is

greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of ENDOX mRNA or protein expression. Alternatively, when expression of ENDOX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of ENDOX mRNA or protein expression. The level of ENDOX mRNA or protein expression in the cells can be determined by methods described herein for detecting ENDOX mRNA or protein.

In yet another aspect of the invention, the ENDOX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with ENDOX ("ENDOX-binding proteins" or "ENDOX-bp") and modulate ENDOX activity. Such ENDOX-binding proteins are also likely to be involved in the propagation of signals by the ENDOX proteins as, for example, upstream or downstream elements of the ENDOX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for ENDOX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an ENDOX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with ENDOX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way

of example, and not of limitation, these sequences can be used to: (*i*) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (*ii*) identify an individual from a minute biological sample (tissue typing); and (*iii*) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the ENDOX sequences, SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or fragments or derivatives thereof, can be used to map the location of the ENDOX genes, respectively, on a chromosome. The mapping of the ENDOX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, ENDOX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the ENDOX sequences. Computer analysis of the ENDOX, sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the ENDOX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. *See, e.g.,* D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the ENDOX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see,* Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.,* in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.,* Egeland, *et al.*, 1987. *Nature*, **325**: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the ENDOX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of

affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The ENDOX sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the ENDOX sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The ENDOX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in

SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining ENDOX protein and/or nucleic acid expression as well as ENDOX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant ENDOX expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, , and the various dyslipidemias., metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with ENDOX protein, nucleic acid expression or activity. For example, mutations in an ENDOX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with ENDOX protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining ENDOX protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of ENDOX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of ENDOX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting ENDOX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes ENDOX protein such that the presence of ENDOX is detected in the biological sample. An agent for detecting ENDOX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to ENDOX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length ENDOX nucleic acid, such as the nucleic acid of SEQ ID NO: 1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to ENDOX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting ENDOX protein is an antibody capable of binding to ENDOX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect ENDOX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of ENDOX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of ENDOX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of ENDOX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of ENDOX protein include introducing into a subject a labeled anti-ENDOX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting ENDOX protein, mRNA, or genomic DNA, such that the presence of ENDOX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of ENDOX protein, mRNA or genomic DNA in the control sample with the presence of ENDOX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of ENDOX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting ENDOX protein or mRNA in a biological sample; means for determining the amount of ENDOX in the sample; and means for comparing the amount of ENDOX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect ENDOX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant ENDOX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with ENDOX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant ENDOX expression or activity in which a test sample is obtained from a subject and ENDOX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of ENDOX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant ENDOX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein,

peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant ENDOX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant ENDOX expression or activity in which a test sample is obtained and ENDOX protein or nucleic acid is detected (*e.g.*, wherein the presence of ENDOX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant ENDOX expression or activity).

The methods of the invention can also be used to detect genetic lesions in an ENDOX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an ENDOX-protein, or the misexpression of the ENDOX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (*i*) a deletion of one or more nucleotides from an ENDOX gene; (*ii*) an addition of one or more nucleotides to an ENDOX gene; (*iii*) a substitution of one or more nucleotides of an ENDOX gene, (*iv*) a chromosomal rearrangement of an ENDOX gene; (*v*) an alteration in the level of a messenger RNA transcript of an ENDOX gene, (*vi*) aberrant modification of an ENDOX gene, such as of the methylation pattern of the genomic DNA, (*vii*) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an ENDOX gene, (*viii*) a non-wild-type level of an ENDOX protein, (*ix*) allelic loss of an ENDOX gene, and (*x*) inappropriate post-translational modification of an ENDOX protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an ENDOX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the ENDOX-gene (*see,* Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682).

This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an ENDOX gene under conditions such that hybridization and amplification of the ENDOX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see,* Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see,* Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Qβ Replicase (*see,* Lizardi, *et al*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an ENDOX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in ENDOX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g.,* Cronin, *et al.*, 1996. *Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in ENDOX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al.*, *supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller,

specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the ENDOX gene and detect mutations by comparing the sequence of the sample ENDOX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.,* Naeve, *et al.*, 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen, *et al.*, 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.*, 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the ENDOX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.,* Myers, *et al.*, 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type ENDOX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S_1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.,* Cotton, *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.*, 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in ENDOX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli*

cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g.,* Hsu, *et al.*, 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an ENDOX sequence, *e.g.*, a wild-type ENDOX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.,* U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in ENDOX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g.,* Orita, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA*: 86: 2766; Cotton, 1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control ENDOX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g.,* Keen, *et al.*, 1991. *Trends Genet.* 7: 5.

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g.,* Myers, *et al.*, 1985. *Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g.,* Rosenbaum and Reissner, 1987. *Biophys. Chem.* 265: 12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g.,* Saiki, *et al.*, 1986. *Nature* 324: 163;

Saiki, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.,* Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.,* Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.,* Gasparini, *et al.,* 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.,* Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an ENDOX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which ENDOX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on ENDOX activity (*e.g.*, ENDOX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, , and the various dyslipidemias., metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases

and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of ENDOX protein, expression of ENDOX nucleic acid, or mutation content of ENDOX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM

show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of ENDOX protein, expression of ENDOX nucleic acid, or mutation content of ENDOX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an ENDOX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of ENDOX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase ENDOX gene expression, protein levels, or upregulate ENDOX activity, can be monitored in clinical trails of subjects exhibiting decreased ENDOX gene expression, protein levels, or downregulated ENDOX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease ENDOX gene expression, protein levels, or downregulate ENDOX activity, can be monitored in clinical trails of subjects exhibiting increased ENDOX gene expression, protein levels, or upregulated ENDOX activity. In such clinical trials, the expression or activity of ENDOX and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including ENDOX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates ENDOX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of ENDOX and other genes implicated in the disorder. The levels of gene

expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of ENDOX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of an ENDOX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the ENDOX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the ENDOX protein, mRNA, or genomic DNA in the pre-administration sample with the ENDOX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of ENDOX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of ENDOX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant ENDOX expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, , and the various dyslipidemias., metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with

Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (*i*) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to an aforementioned peptide; (*iii*) nucleic acids encoding an aforementioned peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endoggenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant ENDOX expression or activity, by administering to the subject an agent that modulates ENDOX expression or at least one ENDOX activity. Subjects at risk for a disease that is caused or contributed to by aberrant ENDOX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation

of symptoms characteristic of the ENDOX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of ENDOX aberrancy, for example, an ENDOX agonist or ENDOX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

Another aspect of the invention pertains to methods of modulating ENDOX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of ENDOX protein activity associated with the cell. An agent that modulates ENDOX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an ENDOX protein, a peptide, an ENDOX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more ENDOX protein activity. Examples of such stimulatory agents include active ENDOX protein and a nucleic acid molecule encoding ENDOX that has been introduced into the cell. In another embodiment, the agent inhibits one or more ENDOX protein activity. Examples of such inhibitory agents include antisense ENDOX nucleic acid molecules and anti-ENDOX antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an ENDOX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, up-regulates or down-regulates) ENDOX expression or activity. In another embodiment, the method involves administering an ENDOX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant ENDOX expression or activity.

Stimulation of ENDOX activity is desirable in situations in which ENDOX is abnormally downregulated and/or in which increased ENDOX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (*e.g.*, preclampsia).

Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The ENDOX nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, , and the various dyslipidemias., metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the ENDOX protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, , and the various dyslipidemias.

Both the novel nucleic acid encoding the ENDOX protein, and the ENDOX protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

EXAMPLES

The following examples illustrate by way of non-limiting example various aspects of the invention.

A family of new human genes, related to the gene encoding human acyl-CoA binding protein (ACBP) / Diazepam- binding Inhibitor (DBI), was identified by an analysis of expressed sequences and genomic DNA sequences. See example 6. The human family consists of 7 novel and 3 known ENDO genes that all contain a highly conserved domain of 20 amino acids. ACBP/DBI is processed to produce a biologically active 18 amino acid peptide (ODN) that influences organismal energy metabolism. It is expected that biological processing of the other family members would lead to other metabolism-regulating peptides.

Synthetic peptides derived from the conserved domain of each human family member, were used in studies of metabolism and to generate polyclonal antibodies in rabbits. The synthetic peptides from the conserved domain of each human and a rat family member were injected into various mouse strains. The injected peptides elicited distinct changes in organismal energy metabolism that effect adipose stores, muscle mass, insulin secretion, glucose utilization and serum lipid levels (triglycerides and cholesterol). See example 1.

Consensus sequences can be derived from the full-length proteins and individual peptides having specific metabolic effects. See example 2 and 3. These human peptides, peptides from non-human species, mutant peptides derived by rational or combinatorial changes based upon the consensus sequences, antibodies and small molecule drugs that interact with the full length proteins, peptides, processing enzymes and/or receptors could have important therapeutic value in the treatment of metabolic disorders. See example 1 and 4 . These disorders include anorexia, cancer-associated cachexia, obesity, Type I and Type II diabetes and the various dyslipidemias.
The invention is also useful as a marker for various cancer types. See example 5

Example 1 Peptide-Elicited Metabolic Effects in AKR Mice

A study was performed to determine the effect of daily ip doses (14 days) of the metabolic regulating peptides (MRPs) derived from the human and rat endozepines on metabolic parameters in AKR (obesity and diabetes prone) and C57Bl/6 (control) mice. This example summarizes the effects of the peptides on AKR mice. Serum cholesterol,

triglycerides, insulin and glucose were monitored along with body weight, body weight change, relative organ weights of liver, reproductive caudal abdominal fat pads, pancreatic and mesenteric tissue weights, quadriceps muscle weight, food and water intake. Histopathologic analysis was performed on selected tissue samples.

Peptides were injected into both AKR and C57Bl/6 mice. The AKR mice are heavier and have higher serum glucose levels (particularly in response to manipulation) than control C57Bl/6 mice. As such, the AKR mice serve as a polygenic model for the forms of obesity and type II diabetes prevalent in the human population. Table 29 summarizes the metabolic effects elicited by each MRP in the AKR mice. MRPs and a peptide with a sequence randomized from the amino acids found in ODN were given by intraperitoneal injection at the indicated dose in 1% DMSO. Control animals were either not manipulated, injected with 1% DMSO or injected with phosphate-buffered saline (PBS). The data in the Table 29 is recorded separately for males and females where indicated and combined for both sexes otherwise. The change from baseline (pre-injection) on serum glucose is reported in mg/dl. The insulin is in microU/ml, the "relative" measures of mesenteric (males and females) fat, uterine fat (females) and quadriceps muscle are recorded as a percent of body weight. The other findings represent consistent findings for both males and females. Statistically significant findings ($p<0.05$) are shaded red (or dark gray) for increases (increased glucose for $MRP1_{18}$, $MRP2_{20}$, $MRP3_{18}$, $MRP4_{18}$, $MRP5_{20}$, $MRP7_{20}$, and $MRP10_{20}$; and increased relative muscle for $MRP1_{20}$ and $MRP10_{20}$) and blue-green (or light gray) for decreases (decreased glucose for $MRP1_{20}$, $MRP4_{20}$ and $MRP6_{20}$; decreased ending insulin for $MRP1_{20}$, $MRP1_{18}$, $MRP2_{20}$, $MRP3_{18}$, $MRP4_{18}$, $MRP8_{20}$, $MRP10_{20}$ and $MRP1_{18}$ (Rat); decreased relative fat ratio for $MRP1_{20}$, $MRP5_{20}$, $MRP10_{20}$ and $MRP1_{18}$ (Rat); and decreased relative muscle for $MRP2_{20}$ and $MRP7_{20}$).

A separate test group was established for each peptide examined, a vehicle control and a non- manipulated group. Each test group consisted of five male and five female AKR mice (10 per peptide). The mice were acclimated for 2 days following shipment. The mice were weighed and divided into groups. Food weight and water volume per cage was measured and recorded.

The mice acclimated another 5 days. On day (-2) pre-injection glucose measurements were made utilizing a glucometer (Johnson & Johnson; One touch Sure Step). Blood samples

were obtained by, first, heating the mice under a heat lamp for approximately 5 minutes before obtaining a drop of blood via the tail vein. Dosing of the various peptides began on day (0). Peptides were prepared identically and dosed at the same concentration (0.05mg/ml in PBS with 1%DMSO, with a dose volume of 10ml/kg) except as noted in Table 29. Stock peptide solutions were prepared by dissolving 2.5 mg of peptide in 0.5 ml of DMSO with 1-2 minutes of sonication, if required, followed by the addition of 0.5ml of sterile PBS to each vial. Dosing solutions were prepared weekly by the addition of 0.5ml of the peptide stock solution to 24.5 mls of sterile PBS. All dose solutions were kept refrigerated throughout the entire study.

The mice were weighed and given daily ip injections of the various peptides or vehicle for 14 consecutive days. Every seven days food weight and water volume was measured and recorded per cage. Blood glucose measurements (via glucometer) were also taken and recorded every 7 days. Blood glucose measurements were taken between 8:00 and 10:00 am and the animals were dosed between 9:00 and 11:00 am daily.

On day 14, one hour prior to necropsy and blood collection, the mice were injected with the final dose of the various peptides or vehicle. Blood (heparinized plasma) was obtained via cardiac or orbital puncture. Plasma chemistries, glucose and insulin, as well as a final glucometer reading were taken at the same time to calibrate/corroborate the glucometer readings. Animals were dissected and placed in formalin for histological sample preparation. Mesenteric fat and pancreas, liver, quadriceps muscle, and caudal abdominal fat pads were removed and weighed for each animal. This study illustrates that the ENDOX peptides can be used to modulate various metabolic functions and treat metabolic disorders.

Table 29

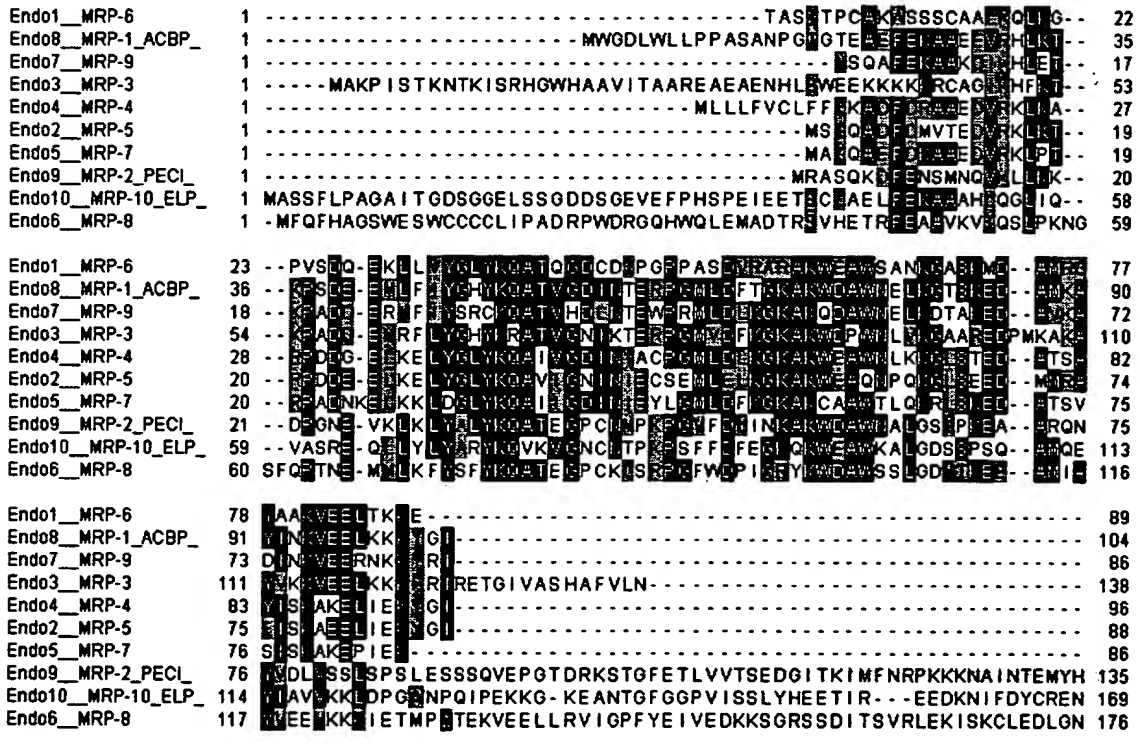
Peptide Name	Dose (mg/kg) (vehicle	Δ Glucose (M./F.)	Ending Insulin (M./F.)	Relative Fat (Mesenteric/ Uterine)	Relative Muscle (M./F.)	Other Findings
$MRP1_{20}$	0.5 DMSO					
$MRP1_{18}$ (ODN)	0.5 DMSO			1.76±0.08 1.44±0.21	0.72±0.02 0.65±0.02	↑cholesterol ↑triglyceride
$MRP2_{20}$	0.5 DMSO			1.72±0.15 1.24±0.25		↓cholesterol ↓triglyceride
$MRP3_{20}$	0.5 PBS	-3.6 (-3%) +32.6	3.70±0.32 4.28±0.98	1.95±0.10 1.74±0.27	0.73±0.03 0.63±0.02	↓Liver
$MRP3_{18}$	0.5 DMSO			2.01±0.04 1.53±0.22	0.66±0.03 0.63±0.02	
$MRP4_{20}$	0.1 DMSO		3.33±0.33 2.40±0.17	2.00±0.16 1.56±0.22	0.76±0.04 0.61±0.05	
$MRP4_{18}$	0.5 DMSO			1.73±0.09 1.16±0.18	0.68±0.04 0.63±0.02	↑cholesterol ↓Liver ↑CPK
$MRP8_{20}$	0.5 DMSO		2.46±0.32 2.40±0.43		0.77±0.07 0.64±0.03	
$MRP6_{20}$	0.5 DMSO		2.95±0.35 2.70±0.80	2.04±0.12 1.42±0.18	0.79±0.07 0.74±0.05	
$MRP7_{20}$	0.5 DMSO		2.35±0.44 1.88±0.51	1.70±0.08 1.47±0.36		↓cholesterol ↓triglyceride ↑CPK ↓Liver
$MRP8_{20}$	0.5 DMSO	+30.6 (+26%)		1.66±0.25 1.99±0.45	0.74±0.06 0.62±0.02	↓cholesterol ↓triglyceride
$MRP9_{20}$	0.5 DMSO	+28.6 (+22%)	4.08±1.72 2.60±0.44	1.74±0.09 1.24±0.14	0.70±0.03 0.67±0.04	↓cholesterol
$MRP10_{20}$	0.5 DMSO					↑↑CPK
$MRP1_{18}$	0.5 DMSO	+7.8 (+6%) +17.2			0.79±0.06 0.71±0.04	
Rando	0.5 DMSO	+49.4 (+40%)	1.42±0.52 0.62±0.12	1.70±0.08 1.70±0.09	0.68±0.03 0.67±0.03	
Vehic	PBS	+8.8 (+6%) +25.1	3.92±0.35 3.58±0.22	1.89±0.15 2.50±0.32	0.70±0.04 0.78±0.05	
Vehic	DMSO	+40.2 (+30%)	2.72±0.62 0.74±0.20	1.77±0.08 1.31±0.15	0.73±0.03 0.63±0.04	

Example 2 Clustal W Alignments

Table 30 shows the Clustal W alignment of all 10 human endozepines. The family consists of two classes of polypeptides – 7 short polypeptides of about 90 amino acids and 3 longer polypeptides containing regions of homology to the other family members at their N termini. There is no homology between the longer forms at their C-termini. This alignment and the phylogenetic distances suggest that the family may have evolved by gene duplications, fusions and independent evolution.

Table 30

Multiple Alignment:



```
Endo1__MRP-6          ***  ------------------------------------------------------------  ***
Endo8__MRP-1_ACBP_    ***  ------------------------------------------------------------  ***
Endo7__MRP-9          ***  ------------------------------------------------------------  ***
Endo3__MRP-3          ***  ------------------------------------------------------------  ***
Endo4__MRP-4          ***  ------------------------------------------------------------  ***
Endo2__MRP-5          ***  ------------------------------------------------------------  ***
Endo5__MRP-7          ***  ------------------------------------------------------------  ***
Endo9__MRP-2_PECI_    136  EIMRALKAASKDDSIITVLTGNGDYYSSGNDLTNFTDIPPGGVEEKAKNNAVLLREFVGC  195
Endo10__MRP-10_ELP_   170  NIDHITKAIKSKNVDVNVKDEEGRALLHWACDRGHKELVTVLLQHRADINCQDNEGQTAL  229
Endo6__MRP-8          177  VLTSTPNAKTVNGKAESSDSGAESEEEEAQEEVKGAEHSDNDKKMMKKSADHKNLEVIVT  236

Endo1__MRP-6          ***  ------------------------------------------------------------  ***
Endo8__MRP-1_ACBP_    ***  ------------------------------------------------------------  ***
Endo7__MRP-9          ***  ------------------------------------------------------------  ***
Endo3__MRP-3          ***  ------------------------------------------------------------  ***
Endo4__MRP-4          ***  ------------------------------------------------------------  ***
Endo2__MRP-5          ***  ------------------------------------------------------------  ***
Endo5__MRP-7          ***  ------------------------------------------------------------  ***
Endo9__MRP-2_PECI_    196  FIDFPKPLIAVVNGPAVGISVTLLGLFDAVYASDRATFHTPFSHLGQSPEGCSSYTFPKI  255
Endo10__MRP-10_ELP_   230  HYASACEFLDIVELLLQSGADPTLRDQDGCLPEEVTGCKTVSLVLQRHTTGKA-------  282
Endo6__MRP-8          237  NGYDKDGFVQDIQNDIHASSSLNGRSTEEVKPIDENLGQTGKSAVCIHQGINDDHVEDVT  296

Endo1__MRP-6          ***  ------------------------------------------------------------  ***
Endo8__MRP-1_ACBP_    ***  ------------------------------------------------------------  ***
Endo7__MRP-9          ***  ------------------------------------------------------------  ***
Endo3__MRP-3          ***  ------------------------------------------------------------  ***
Endo4__MRP-4          ***  ------------------------------------------------------------  ***
Endo2__MRP-5          ***  ------------------------------------------------------------  ***
Endo5__MRP-7          ***  ------------------------------------------------------------  ***
Endo9__MRP-2_PECI_    256  MSPAKATEMLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNALRISKE  315
Endo10__MRP-10_ELP_   ***  ------------------------------------------------------------  ***
Endo6__MRP-8          297  GIQHLTSDSDSE-VYCDSMEQFGQEESLDSFTSNNGPFQYYLGGHSSQPMENSGFREDIQ  355

Endo1__MRP-6          ***  ------------------------------------------------------------  ***
Endo8__MRP-1_ACBP_    ***  ------------------------------------------------------------  ***
Endo7__MRP-9          ***  ------------------------------------------------------------  ***
Endo3__MRP-3          ***  ------------------------------------------------------------  ***
Endo4__MRP-4          ***  ------------------------------------------------------------  ***
Endo2__MRP-5          ***  ------------------------------------------------------------  ***
Endo5__MRP-7          ***  ------------------------------------------------------------  ***
Endo9__MRP-2_PECI_    316  VIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL----------------  359
Endo10__MRP-10_ELP_   ***  ------------------------------------------------------------  ***
Endo6__MRP-8          356  VPPGNGNIGNMQVVAVEGKGEVKHGGEDGRNNSGAPHREKRGGETDEFSNVRRGRGHRMQ  415

Endo1__MRP-6          ***  ------------------------------------------------------------  ***
Endo8__MRP-1_ACBP_    ***  ------------------------------------------------------------  ***
Endo7__MRP-9          ***  ------------------------------------------------------------  ***
Endo3__MRP-3          ***  ------------------------------------------------------------  ***
Endo4__MRP-4          ***  ------------------------------------------------------------  ***
Endo2__MRP-5          ***  ------------------------------------------------------------  ***
Endo5__MRP-7          ***  ------------------------------------------------------------  ***
Endo9__MRP-2_PECI_    ***  ------------------------------------------------------------  ***
Endo10__MRP-10_ELP_   ***  ------------------------------------------------------------  ***
Endo6__MRP-8          416  HLSEGTKGRQVGSGGDGERWGSDRGSRGSLNEQIALVLMRLQEDMQNVLQRLQKLETLTA  475

Endo1__MRP-6          ***  ----------------------------------------------------------    ***
Endo8__MRP-1_ACBP_    ***  ----------------------------------------------------------    ***
Endo7__MRP-9          ***  ----------------------------------------------------------    ***
Endo3__MRP-3          ***  ----------------------------------------------------------    ***
Endo4__MRP-4          ***  ----------------------------------------------------------    ***
Endo2__MRP-5          ***  ----------------------------------------------------------    ***
Endo5__MRP-7          ***  ----------------------------------------------------------    ***
Endo9__MRP-2_PECI_    ***  ----------------------------------------------------------    ***
Endo10__MRP-10_ELP_   ***  ----------------------------------------------------------    ***
Endo6__MRP-8          476  AKSSTSTLQTAPQPTSSQRPSWWPFEMSPGVLTFAIIWPFIAQWLVYLYYQRRRR      530
```

Table 30-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 30.

Table 30-1

SEQUENCE IDENTIFIER	SEQ ID NO
Endo1_MRP-6	SEQ ID NO:98
Endo8_MRP-1_ACBP_	SEQ ID NO: 99
Endo7_MRP-9	SEQ ID NO: 100
Endo3_MRP-3	SEQ ID NO: 101
Endo4_MRP-4	SEQ ID NO: 102
Endo2_MRP-5	SEQ ID NO: 103
Endo5_MRP-7	SEQ ID NO: 104
Endo9_MRP-2_PECI_	SEQ ID NO: 106
Endo10_MRP-10_ELP_	SEQ ID NO: 105
Endo6_MRP-8	SEQ ID NO: 107

Example 3 Consensus Sequences for the Metabolism – Regulating Peptides

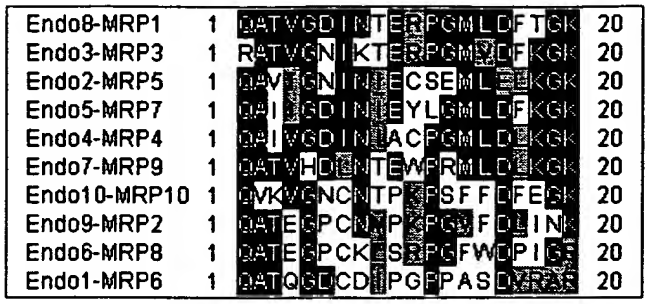
All of the MRPs and several subsets of peptides, that have similar metabolic effects in AKR mice were identified in Table 29. These can be aligned to identify consensus sequences which are identified in Table 31. The general and preferred consensus sequences are deduced by inspection. The most conserved amino acid at each position in the 20 amino acid motif is in bold print. If that amino acid is invariant in a particular metabolic subset then it is also underlined. If the amino acid at a particular position is highly variable it is represented by an "X." When a small number of amino acids are found at a particular position then they are enclosed within [brackets]. The "general" consensus allows for the most variability in a peptide sequence that could have similar metabolic effects. The "preferred" consensus sequence is limited to the amino acids found in the inspected peptide subset. Particular subsets of peptides were identified that are associated with cholesterol-lowering properties, fat mass reducing, insulin-lowering, glucose lowering, glucose-raising and muscle mass building properties.

Table 30-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 31.

Table 31

Consensus Sequences for the Metabolism – Regulating Peptides

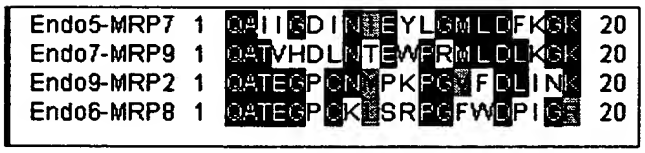
Global Alignment:



Global Consensus Sequence:

QAT[V/I/E]G[D/N/P][I/L/C][N/K][I/L/M/T][E/S/P][K/R]PGMLD[L/F]KG[K/R] (SEQ ID NO:37)

Cholesterol – Lowering Peptides:



Cholesterol-Lowering Consensus Sequence:

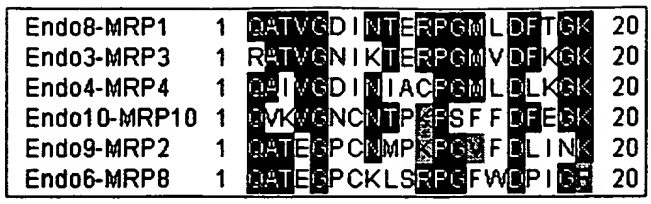
General **QAT[E/V/I]G[D/P][C/I/L][N/K]X[E/X]XP[G/R][M/X[[L/X]D[L/X]XG[K/R]** (SEQ ID NO:38)
Preferred**QATEG**[DP]C[KRN][AITVFLM]X[KR]**PG**[AITVFLM][WAITVFLM]**D**[PAITVFLM]IX[KR] (SEQ ID NO:39)

Adipose – Lowering Peptides:

Adipose-Lowering Consensus Sequence:

General **QAX[VI]GNIN[T/I]EXPXML[DE]FXGK** (SEQ ID NO:40)
Preferred **Q**[AITVFLM]X[AITVFLM]**G**[DEN]X**N**X**E**XXX [AITVFLM][DE]XX**GK** (SEQ ID NO:41)

Insulin – Lowering Peptides:



Insulin-Lowering Consensus Sequence:

QATV<u>G</u>[D/N/P][I/C]N[T/I/M/L]X[R/K]<u>P</u>G[M/X]X<u>D</u>[F/L]XG[K/R] (SEQ ID NO:42)

Glucose – Lowering Peptides:

Glucose-Lowering Consensus Sequence:

<u>QA</u>TV<u>GD</u>[I/C]NIXX<u>P</u>[G/P][M/A][L/S]<u>D</u>XX[G/A][K/R] (SEQ ID NO:43)

Glucose – Raising Peptides:

Glucose-Raising Consensus Sequence:

QAXX<u>G</u>[N/D/P][I/C]N[T/I/M][E/P]X[P/X][G/X][M/X]X<u>D</u>[F/L][K/X]<u>GK</u> (SEQ ID NO:44)

Muscle Mass Raising Peptides:

Muscle-Mass Raising Consensus Sequence:

General **<u>QXXVGXXNTX</u>(R/K)<u>P</u><u>XXXDFXGK</u>** (SEQ ID NO:148)

Preferred **<u>Q</u>**[AITVFLM]X**<u>VG</u>**[DEN]X**<u>NTX</u>**[RK]**<u>P</u>**XX[AITVFLM]**<u>DF</u>**X**<u>GK</u> (SEQ ID NO:45)

Example 4 Effects on Mesenteric Adipocyte Size

Figure 1 consists of 6 photomicrographs of mesenteric adipose fixed from male AKR mice after treatment with the agent indicated beneath each panel. In the top panels (A-C) large adipocytes predominate whereas in the bottom panels (D-F) smaller adipocytes predominate. This correlates with the decrease in the mass of that particular adipose deposit in response to the specific treatment. There are subsets of MRPs that affect the mass of specific adipose deposits and subsets of MRPs that have influence on other metabolic parameters.

Example 5 Gene Expression, Cloning & Antibody Production Summary

Table 32 lists the members of the endozepine family (EndoX) and the corresponding bio-active Metabolism-Regulating Peptide (MRP-#) synthesized from each endozepine. In addition, the tissue(s) where the gene is most highly expressed is indicated from the result of real-time quantitative RTQ-PCR (TaqMan) using total RNA as well as by traditional PCR using ds cDNA as a template. PCR products corresponding to the coding regions of the various endozepines have been amplified for cloning purposes as noted. Anti-peptide antibodies have been generated in rabbits for each human synthetic 20 amino acid peptide. Anti-peptide titers that have been determined (ELISA) are indicated.

Table 32

Endozepine Name	Bio-Active Peptide Name	Bio-Active Peptide Sequence	Gene Expression Distribution				Polyclonal Antisera Production
			TaqMan - Principal Tissues		Traditional RT-PCR		
		20 - mers	Normal Tissues	Cancer Tissues	Normal Tissue Distribution	Physical Product For Cloning or Cloned	Highest Titer
Endo1	MRP-6	QATQGDCDIPGPPASDVRAR (SEQ ID NO:117)	Liver Endothelial cells	Multiple CAs	Brain Heart Liver Pancreas Skeletal Muscle Small Intestine	Yes	Antibody Available
Endo2	MRP-5	QAVIGNINIECSEMLELKGK (SEQ ID NO:110)	Brain Pancreas	Colon CA Lung CA	Adipose Bone Marrow Fetal Brain Fetal Liver	Yes	Antibody Available
Endo3	MRP-3	RATVGNIKTERPGMVDFKGK (SEQ ID NO:109)	Adipose Skeletal Muscle	Breast CA	Adipose Brain Liver Heart Pancreas Skeletal Muscle Small Intestine	Yes	2,000
Endo4	MRP-4	QAIVGDINIACPGMLDLKGK (SEQ ID NO:112)	Hematopoietic	Low Expression	Liver	Yes	Antibody Available
Endo5	MRP-7	QAIIGDINIEYLGMLDFKGK (SEQ ID NO:111)					Antibody Available
Endo6	MRP-8	QATEGPCKLSRPGFWDPIGR (SEQ ID NO:116)	Skeletal Muscle	Multiple CAs	Brain Liver Muscle	Yes	13,000
Endo7	MRP-9	QATVHDLNTEWPRMLDLKGK (SEQ ID NO:113)	Adipose	Liver CA	Adip. Fetal Brain Fetal Liver	Yes	12,000
Endo8 (ACBP / DBI / CCK-RP)	MRP-1	QATVGDINTERPGMLDFTGK (SEQ ID NO:108)	Heart Skeletal Muscle Liver Endothelial cells	Breast CA Colon CA Melanoma	Brain Liver	Yes	14,000
Endo9 (PECI) (D2,D3 Enoyl CoA Isomerase)	MRP-2	QATEGPCNMPKPGVFDLINK (SEQ ID NO:115)			Brain Liver Skeletal Muscle	Yes	3,600
Endo10 (ELP)	MRP-10	QVKVGNCNTPKPSFFDFEGK (SEQ ID NO:114)			Fetal Brain Fetal Liver Bone Marrow	Yes	10,000
		18 - mers					
Endo8 (ACBP / DBI / CCK-RP)	MRP-1s (ODN)	QATVGDINTERPGMLDFT-- (SEQ ID NO:118)					
Rat ODN	MRP-Rn. ODN	QATVGDVNTDRPGLLDLK-- (SEQ ID NO:119)					
Endo3	MRP-3s	RATVGNIKTERPGMVDFK-- (SEQ ID NO:120)					

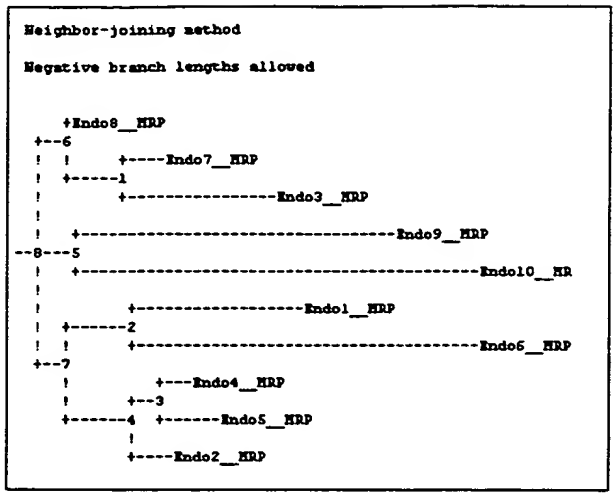
Example 6 Phylogenetic Relationships Among the Novel and Known Endozepines

Table 33 indicates the relatedness of the various full-length endozepines as well as the MRPs encoded within each endozepine. The distances are computed by the program "Phylip" that

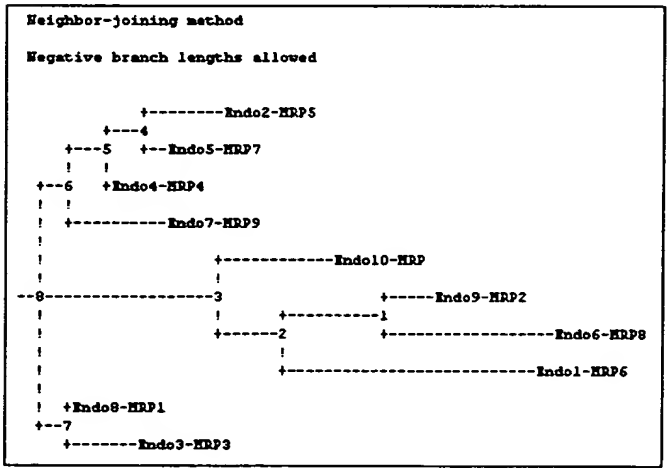
calculates neighbor-joining distances, a method for describing the relative relatedness of the input sequences.

Table 33

Full Protein



Metabolism – Regulating Peptide Sequence Only



Example 7 Identification of Related Peptides In Other Proteins

Tables 34 and 35 illustrate how the specific consensus motifs can be used to identify polypeptides in other animal species that may be involved in the regulation of the same metabolic parameter. The GenBank database was searched using two general consensus sequences, the first associated with muscle mass building (Table 34) and the second associated with fat mass reduction (Table 35). The consensus sequences identified in each peptide are

shown in bold font. Many polypeptides from multiple species were identified with 0, 1 or 2 mismatches from the consensus sequence. The highest similarity is seen in ACBPs from other species. This is also seen in Figure 1, which illustrates that orthologous sequences can be found for 6 of the 10 members of the human endozepine family.

TABLE 34

Muscle-Raising Motif

Pattern Submitted: *'qxxvgxxntx[kr]pxxxdfxgk'* (SEQ ID NO:46)
Database Analyzed: *Non-Redundant Composite* *
Matching Sequences To Show: *Top 50*
Mismatches Allowed: *3*
Allow Insertions/Deletions: *no*
Allow matches to database ambiguities: *no*

Database contains 553,883 sequences.
Reporting all 29 matching sequences (did not trigger cutoff of 50 matches).
There are 4 equivalence classes of equally good matches.

SWISSPROT-ACC:P07107 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) - Bos taurus (Bovine), 86 aa.

# Mismatches	Match Position	Match Context
0	33-52	IYSHYK**QATVGDINTERPGMLDFKGKAKWDAW** (SEQ ID NO:121)

SWISSPROT-ACC:P07108 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) - Homo sapiens (Human), 86 aa.

# Mismatches	Match Position	Match Context
0	33-52	IYGHYK**QATVGDINTERPGMLDFTGKAKWDAW** (SEQ ID NO:122)

SPTREMBL-ACC:Q9VLS4 CG8498 PROTEIN - Drosophila melanogaster (Fruit fly), 90 aa.

# Mismatches	Match Position	Match Context
0	35-54	LYSLYK**QATVGDCNTDKPGFLDFKGKAKWEAW** (SEQ ID NO:123)

SPTREMBL-ACC:Q9PRL8 ACYL-COENZYME A BINDING PROTEIN, ACBP - Gallus gallus (Chicken), 86 aa.

# Mismatches	Match Position	Match Context
0	33-52	VYSHYK**QATVGDVNTDRPGMLDFKGKAKWDAW** (SEQ ID NO:124)

REMTREMBL-ACC:CAA44618 ACYL-COA-BINDING PROTEIN /DIAZEPAM-BINDING INHIBITOR - synthetic construct, 87aa

# Mismatches	Match Position	Match Context
0	34-53	IYSHYK**QATVGDINTERPGMLDFKGKAKWDAW** (SEQ ID NO:125)

pir-id:NZHU endozepine [validated] - human, 87 aa.

# Mismatches	Match Position	Match Context

0 34-53 IYGHYKQATVGDINTERPGMLDFTGKAKWDAW (SEQ ID NO:126)

pir-id:S63593 acyl-coenzyme A-binding protein - turtle, 86 aa.

Mismatches Match Position Match Context
0 33-52 IYSHFKQATVGDINTERPGFLDFKGKAKWDAW (SEQ ID NO:127)

pir-id:S63594 acyl-coenzyme A-binding protein - mallard, 86 aa.

Mismatches Match Position Match Context
0 33-52 VYSHYKQATVGDVNTDRPGMLDFKGKAKWDAW (SEQ ID NO:128)

P31786 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) - Mus musculus (Mouse), 86 aa.
Mismatches Match Position Match Context
1 33-52 IYSHFKQATVGDVNTDRPGLLDLKGKAKWDSW (SEQ ID NO:129)

SWISSPROT-ACC:P12026 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) [CONTAINS: DBI(32-86)] - Sus scrofa (Pig), 86 aa.
Mismatches Match Position Match Context
1 33-52 IYSHYKQATVGDINTERPGILDLKGKAKWDAW (SEQ ID NO:130)

Table 35

Identification of Related Peptides In Other Proteins

Adipose-Lowering Motif

Pattern Submitted: 'qax[vi]gnin[ti]expxml[de]fxgk' (SEQ ID NO:40)
Database Analyzed: Non-Redundant Composite *
Matching Sequences To Show: Top 50
Mismatches Allowed: 3
Allow Insertions/Deletions: no
Allow matches to database ambiguities: no

Database contains 553,883 sequences.
Reporting all 14 matching sequences (did not trigger cutoff of 50 matches).
There are 3 equivalence classes of equally good matches.

SWISSPROT-ACC:P07107 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) - Bos taurus (Bovine), 86 aa.

Mismatches Match Position Match Context
1 33-52 IYSHYKQATVGDINTERPGMLDFKGKAKWDAW (SEQ ID NO:131)

SWISSPROT-ACC:P07108 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) - Homo sapiens (Human), 86 aa.

Mismatches Match Position Match Context
1 33-52 IYGHYKQATVGDINTERPGMLDFTGKAKWDAW (SEQ ID NO:132)

REMTREMBL-ACC:CAA44618 ACYL-COA-BINDING PROTEIN /DIAZEPAM-BINDING INHIBITOR - synthetic construct,87aaaa.

# Mismatches	Match Position	Match Context
1 3	4-53	IYSHYK**QATVGDINTERPGMLDFKGKAKWDAW** (SEQ ID NO:133)

pir-id:NZHU endozepine [validated] - human, 87 aa.

# Mismatches	Match Position	Match Context
1	34-53	IYGHYK**QATVGDINTERPGMLDFTGKAKWDAW** (SEQ ID NO:134)

SWISSPROT-ACC:P45882 ACYL-COA-BINDING PROTEIN (ACBP) (DIAZEPAM BINDING INHIBITOR) (DBI) (ENDOZEPINE) (EP) - Anas platyrhynchos (Domestic duck), 103 aa.

# Mismatches	Match Position	Match Context
2	50-69	LYGFYK**QATVGDINIECPGMLDLKGKAKWEAW** (SEQ ID NO:135)

pir-id:S63593 acyl-coenzyme A-binding protein - turtle, 86 aa.

# Mismatches	Match Position	Match Context
2	33-52	IYSHFK**QATVGDINTERPGFLDFKGKAKWDAW** (SEQ ID NO:136)

Example 8 Orthologous Gene Products in Multiple Species

Table 36 illustrates the high degree of conservation of the MRP motifs across diverse species. The human MRP sequences were used to search GenBank for related sequences. Eleven other sequences with a high degree of similarity were identified. Using a larger peptide centered on the 20 amino acid MRP domain will identify additional polypeptides. The 11 entries from other species are represented by their accession numbers. The indicated set of 21 peptides from species ranging from frogs to humans was examined for their relationship to the set of 10 human peptides by the PHYLIP, PILEUP and Clustal W algorithms. As can be seen in Table 36, there are non-human peptides that are more like some human sequences than other human sequences. This suggests that the peptide-containing genes arose and followed divergent paths in energy metabolism early in evolution.

Table 36

Orthologous Gene Products in Multiple Species

PHYLIP

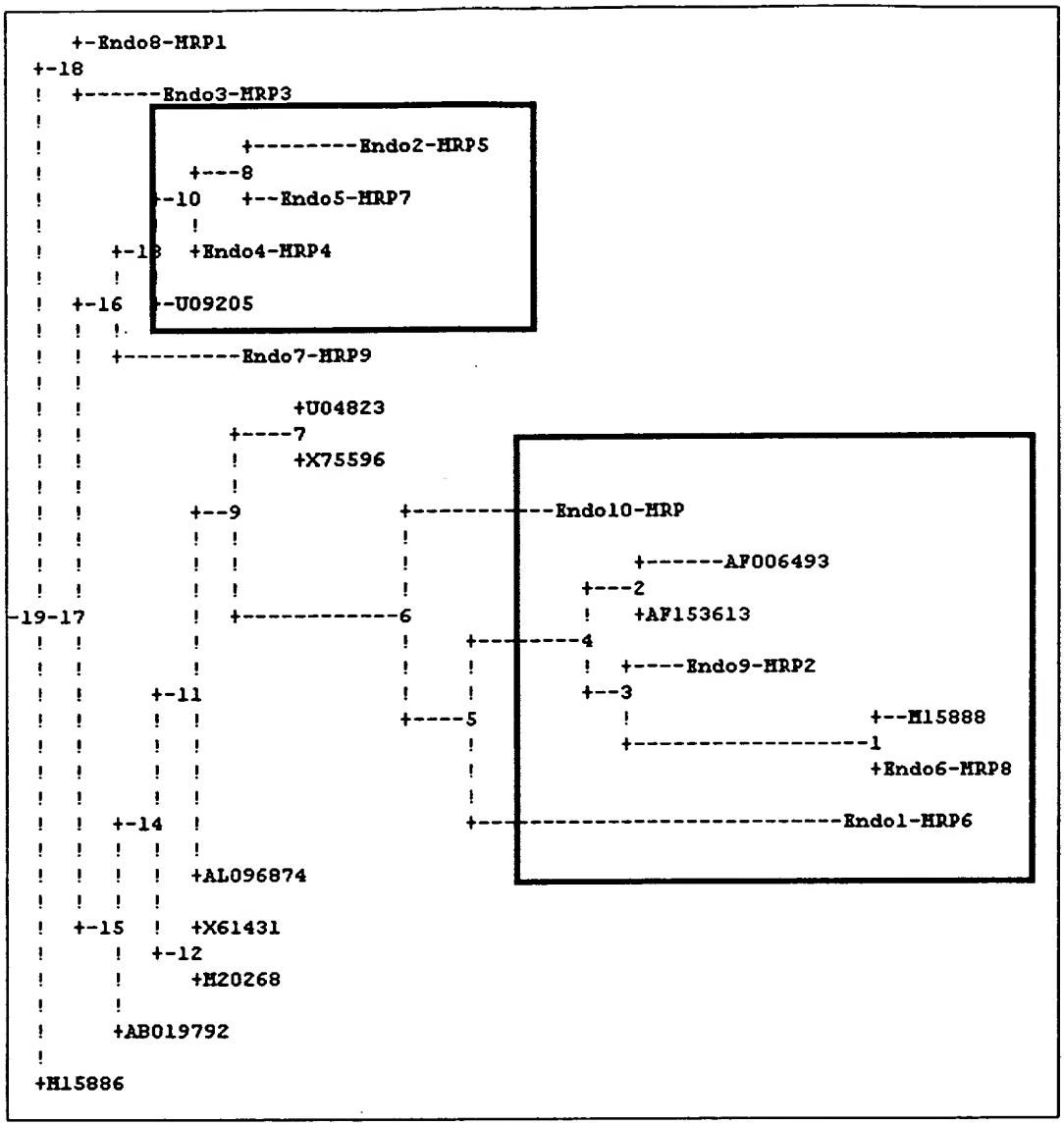
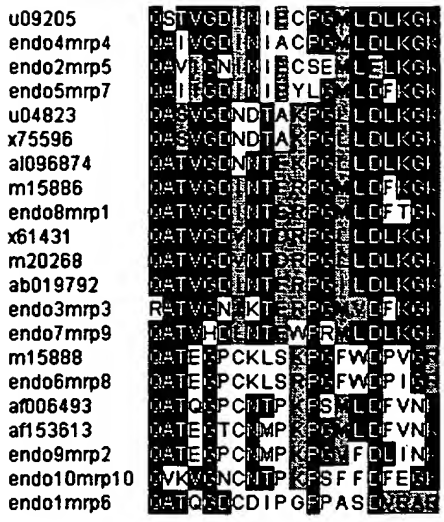


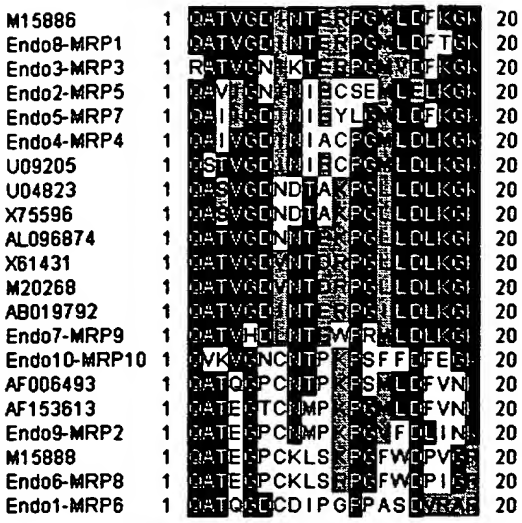
Table 37 also illustrates the high degree of conservation of the MRP motifs across diverse species.

Table 37

PILEUP



CLUSTAL W



5

Table 37-1 lists the sequence identifiers and sequence identification numbers (SEQ ID NO) for the sequences displayed in Table 37.

Table 37-1

SEQUENCE IDENTIFIER	SEQ ID NO
U09205	SEQ ID NO: 137
Endo4-MRP4	SEQ ID NO: 112
Endo2-MRP5	SEQ ID NO: 110
Endo5-MRP7	SEQ ID NO: 111
U04823	SEQ ID NO: 138
X75596	SEQ ID NO: 139
AL096874	SEQ ID NO: 140
M15886	SEQ ID NO: 141
Endo8-MRP1	SEQ ID NO: 108
X61431	SEQ ID NO: 142
M20268	SEQ ID NO: 143
AB019792	SEQ ID NO: 144
Endo3-MRP3	SEQ ID NO: 109
Endo7-MRP9	SEQ ID NO: 113
M15888	SEQ ID NO: 145

Endo6-MRP8	SEQ ID NO: 116
AF006493	SEQ ID NO: 146
AF153613	SEQ ID NO: 147
Endo9-MRP2	SEQ ID NO: 115
Endo10-MRP10	SEQ ID NO: 114
Endo1-MRP6	SEQ ID NO: 117

Example 9 PCR Products Of Endozepine Coding Sequences

The coding sequence of each human endozepine was cloned into numerous expression vectors in order to examine the complex ways in which these secreted proteins and their biologically active MRPs affect energy metabolism. Some of the cDNAs have been cloned in bacteria in order to prepare recombinant proteins. The complete set of proteins were expressed in bacteria, yeast and mammalian cells to study their individual biochemical activities, protein-protein interactions and how they modulate energy metabolism in isolated cells or whole animals. Physical DNA molecules for this purpose were generated by PCR from the various tissues, noted above in Table 29, and can be seen on this ethidium bromide-stained agarose gel (Figure 2). The individual PCR products for Endos 1-4, and Endos 6-10 are of the expected size as deduced from the sequences (including 42 bp of flanking cloning sequence here).

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49;

(b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;

(c) an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49; and

(d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence.

2 The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49.

3. The polypeptide of claim 2, wherein said allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48.

4. The polypeptide of claim 1, wherein the amino acid sequence of said variant comprises a conservative amino acid substitution.

5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49;

(b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;

(c) an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49;

(d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence;

(e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence chosen from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; and

(f) a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).

6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.

7. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.

8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48.

9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48;

(b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, provided that no more than 20% of the nucleotides differ from said nucleotide sequence;

(c) a nucleic acid fragment of (a); and

(d) a nucleic acid fragment of (b).

10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to a nucleotide sequence chosen from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 22, 25, 28, 31, 34, 46, and 48, or a complement of said nucleotide sequence.

11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 20% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;

(b) an isolated second polynucleotide that is a complement of the first polynucleotide; and

(c) a nucleic acid fragment of (a) or (b).

12. A vector comprising the nucleic acid molecule of claim 11.

13. The vector of claim 12, further comprising a promoter operably-linked to said nucleic acid molecule.

14. A cell comprising the vector of claim 12.

15. An antibody that binds immunospecifically to the polypeptide of claim 1.

16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.

17. The antibody of claim 15, wherein the antibody is a humanized antibody.

18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:

(a) providing the sample;

(b) contacting the sample with an antibody that binds immunospecifically to the polypeptide; and

(c) determining the presence or amount of antibody bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.

19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:

(a) providing the sample;

(b) contacting the sample with a probe that binds to said nucleic acid molecule; and

(c) determining the presence or amount of the probe bound to said nucleic acid molecule, thereby determining the presence or amount of the nucleic acid molecule in said sample.

20. The method of claim 19 wherein presence or amount of the nucleic acid molecule is used as a marker for cell or tissue type.

21. The method of claim 20 wherein the cell or tissue type is cancerous.

22. A method of identifying an agent that binds to a polypeptide of claim 1, the method comprising:

(a) contacting said polypeptide with said agent; and

(b) determining whether said agent binds to said polypeptide.

23. The method of claim 22 wherein the agent is a cellular receptor or a downstream effector.

24. A method for identifying an agent that modulates the expression or activity of the polypeptide of claim 1, the method comprising:

(a) providing a cell expressing said polypeptide;

(b) contacting the cell with said agent, and

(c) determining whether the agent modulates expression or activity of said polypeptide, whereby an alteration in expression or activity of said peptide indicates said agent modulates expression or activity of said polypeptide.

25. A method for modulating the activity of the polypeptide of claim 1, the method comprising contacting a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

26. A method of treating or preventing a ENDOX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the polypeptide of claim 1 in an amount sufficient to treat or prevent said ENDOX-associated disorder in said subject.

27. The method of claim 26 wherein the disorder is selected from the group consisting of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, cancers, cancer-associated cachexia, and dyslipidemia.

28. The method of claim 26 wherein the disorder is related to organismal energy metabolism that effect adipose stores, muscle mass, insulin secretion, glucose utilization and serum lipid levels including triglycerides and cholesterol

29. The method of claim 26, wherein said subject is a human.

30. A method of treating or preventing a ENDOX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the nucleic acid of claim 5 in an amount sufficient to treat or prevent said ENDOX-associated disorder in said subject.

31. The method of claim 30 wherein the disorder is selected from the group consisting of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, cancers, cancer-associated cachexia, and dyslipidemia.

32. The method of claim 30 wherein the disorder is related to organismal energy metabolism that effects adipose stores, muscle mass, insulin secretion, glucose utilization and serum lipid levels including, triglycerides and cholesterol

32. The method of claim 30, wherein said subject is a human.

34. A method of treating or preventing a ENDOX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the antibody of claim 15 in an amount sufficient to treat or prevent said ENDOX-associated disorder in said subject

35. The method of claim 34 wherein the disorder is selected from the group consisting of diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, cancers, cancer-associated cachexia, and dyslipidemia.

36. The method of claim 34 wherein the disorder is related to organismal energy metabolism that effects adipose stores, muscle mass, insulin secretion, glucose utilization and serum lipid levels including, triglycerides and cholesterol

37. The method of claim 34, wherein the subject is a human.

38. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically-acceptable carrier.

39. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically-acceptable carrier.

40. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically-acceptable carrier.

41. A kit comprising in one or more containers, the pharmaceutical composition of claim 38.

42. A kit comprising in one or more containers, the pharmaceutical composition of claim 39.

43. A kit comprising in one or more containers, the pharmaceutical composition of claim 40.

44. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:

(a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and

(b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease;

wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.

45. The method of claim 44 wherein the predisposition is to cancers.

46. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:

(a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and

(b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;

wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.

47. The method of claim 46 wherein the predisposition is to cancers.

48. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising an amino acid sequence of at least one of SEQ ID NO:2, 4, 6, 8, 10, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35 to 45 inclusive, 47, and 49, or a biologically active fragment thereof.

49. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.

ENDOZEPINE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES ENCODING THEM

ABSTRACT

Disclosed herein are novel human nucleic acid sequences which encode endozepine-like polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies which immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving this novel human endozepine-like nucleic acid and protein.